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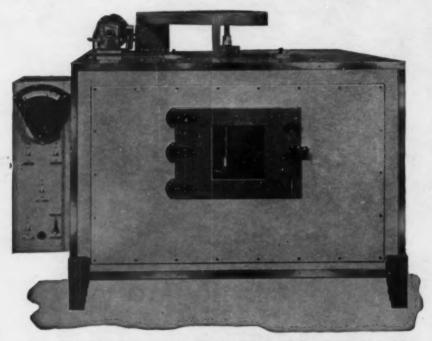
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No. 6

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No. 6

STUDIES ON THE EFFECT OF FLOUR-FRACTION INTERCHANGE UPON CAKE QUALITY¹

D. H. DONELSON AND J. T. WILSON²

ABSTRACT

A good-quality unbleached cake flour and a poor-quality cake flour were fractionated into water-solubles, gluten, starch tailings, and prime starch. Fractions were combined to form reconstituted flours comprising complete interchanges of the components at the composition of each flour. These reconstituted flours were bleached, and layer cakes were baked using a lean (no milk or eggs, high sugar) formulation.

The contributions to cake volume of the gluten, water-solubles, and tailings fractions obtained from the good flour were significantly greater than, and the effects on gross cake structure with these fractions were superior to, those of the corresponding fractions obtained from the poor flour. The prime starch from the poor flour was significantly superior to that of the good flour. Gluten had the greatest effect on cake volume and structure. Interactions of gluten × composition and water-solubles × tailings × composition were highly significant, which indicated considerable variation of the responses to these flour components with concentration.

It was suggested that the expression of these quality factors may be contingent upon an inherent quality aspect, related to the physical and chemical constitution of each factor, and upon a concentration dependence, related to the percent composition of the flour.

A useful procedure in the investigation of soft wheat quality is that of baking lean-formula cakes from reconstituted test flours. For analytical work, the lean formulation developed at the Wooster laboratory for the baking of white layer cakes has several advantages. The omission of milk and eggs from the formula eliminates all structure-forming elements except the flour, and the high sugar content acts as a tenderizing agent. Consequently, maximum stress is placed upon the flour, and variation found in finished cake may be more readily associated with the treatments used.

¹ Manuscript received October 12, 1959. Contribution from the Soft Wheat Quality Laboratory, Ohio Agricultural Experiment Station, Wooster, Ohio. A report of the NC-30 research project of the North Central States Soft Wheat Technical Committee. Presented in part at Cincinnati Section Meeting, AACC, Cincinnati, Ohio. Aaril 11, 1952.

Previous work (2) demonstrated that white layer cake quality, as measured by volume and appearance, may depend upon the composition or relative proportions of the component flour fractions. The quality of cakes baked from flour fractions obtained from a good soft wheat cake flour could be made to vary considerably simply by altering the proportions of fractions. The effect that each component had depended not only upon the amount of it present, but also upon the amounts of the other fractions used, which suggested that flour quality depends in part upon a balance of its components. For that work, quality of flour fractions was held constant by taking them from the same source, and the proportions of the components were varied by making up synthetic flours containing different amounts of each fraction.

Implicit in such an analytical procedure is the assumption that the quality effect, Q_i , of each fraction is a function of an *inherent quality*, q_i , and a *concentration dependence*, c_i , i.e., $Q_i = f(q_i, c_i)$, analogous to a vector, which has both magnitude and direction. Fraction quality evaluation, then, would require exploration of both inherent quality and concentration. Over-all cake quality, \overline{Q} , would be a summation of the quality contributions of all fractions, so that

 $\overline{Q} = \sum_{i} Q_i = \sum_{i} f(q_i, c_i)$. This is subject to the constraint that $\sum_{i} c_i = 100$,

where the c_i are expressed as percentages of the fractions in the flour. For the aforementioned work, all the q_i were held constant and the c_i were varied. Changes in \overline{Q} showed that for the flour used, concentration dependence was significant.

In order to test the other half of this postulate, it would be desirable to hold the flour composition constant and alter or vary the inherent quality of each flour fraction to gain measures of the effect of inherent fraction quality on over-all cake quality. This report is a presentation of data from such an extension of the earlier work, and is concerned, therefore, with the relative quality of flour components.

A systematic substitution of flour fractions obtained from a poorgrade cake flour for those of a good cake flour might allow inferences to be made concerning the contribution of each component to the over-all quality of the cake and indicate how the factors within a flour determine its property of being good or poor. (We select a good and a poor flour for the first experiment in order to ensure that differences in relative quality are bound to be significant.) If we determine by fractionation the good flour composition, C, then by combining good and poor fractions in various ways (all reconstituted flours with the same composition, C), we should obtain measures of the relative inherent quality of the pairs of fractions. However, assuming that the quality expression of each factor is a function of both q and c, the use of a single composition leads to confounding of inherent quality and concentration dependence, since we have no way of estimating concentration dependence from just one composition. If, for composition C, we find that a fraction from the poor flour is poorer than its counterpart, it is entirely possible that for another composition, C', the positions would be reversed. Lest this appear purely academic, consider that from one point of view, flour milling, especially some of the newer techniques (3), can be considered to be just preparation of products whose components are present in certain concentrations. It becomes a matter of practical importance to discover how greatly flour quality might depend on flour composition.

The smallest number of compositions that might be used to gain some estimate of the effect of concentration on substitutions to test fraction inherent quality is two. A large number, so that one could prepare response surfaces as was done in the previous work (2), would be preferred, but the experimental complications involved in substituting flour fractions at each of a number of compositions are appalling. An experimental design that offers some estimation of concentration dependence and relative inherent quality is one in which substitutions of components from the poor flour into the good one at composition C of the good flour is accompanied by the converse, substitution of good flour fractions into the poor flour at the composition C' of the poor flour. With such a design, we obtain data on the quality contributions of components from two flours at compositions of both flours, and gain thereby some measure of inherent quality, q, of each fraction and an inkling of the effect of concentration, c, on its quality at two levels of concentration. We can, if there are differences, at least rank the pairs of fractions for two concentrations and see whether concentration has any effect on their ranking.

An appropriate experimental method is obviously a factorial, especially since we assume that over-all cake quality is, in a broad sense, a summation of flour component contributions to that quality. The experimental procedure would then be a complete interchange of tractions at each composition level. A similar interchange procedure was used by Zaehringer et al. (8) for an experiment using biscuits as the baked product.

Materials and Methods

A 48% extraction, unbleached, commercially milled soft wheat

flour (hereafter designated for convenience commercial) was selected as the good-quality cake flour. An inferior cake flour (designated Pawnee) was obtained by milling a sample of Pawnee wheat to 50% extraction. This flour was prepared on Allis-Chalmers laboratory equipment, then Raymond-milled to obtain particle size comparable to that of the commercial flour. The protein content of the commercial flour was 7.6%, and that of the Pawnee flour was 10.1%, on a 14% moisture basis.

Each flour was fractionated into water-solubles, gluten, starch tailings, and prime starch by a batter and screening method modified slightly from that described in the previous report (2). With 1500-g. batches of flour, a small residue remained on the screen after removal of gluten. It appeared to be composed of hydrated tailings particles too large to pass the 25 standard silk bolting cloth (0.0025-in. opening), along with a quantity of dispersed gluten and protein foam. As an additional step, the residues from several batches were pooled, triturated with water, and centrifuged; the supernatant was added to the water-solubles fraction. Gluten was removed from the residue by kneading in a small quantity of water, and added to the main gluten fraction. The remainder of the material was combined with the tailings. All fractions were lyophilized, ground at the fine setting of a Hobart coffee mill (except water-solubles, which were put through at an open setting), and stored under refrigeration.

With the above method, fractions totaling 96.0% of the commercial flour and 97.5% of the Pawnee flour were recovered. Table I reports the relative proportions of fractions, their protein content, and the percentages of total flour protein in each fraction, as found

TABLE I
PERCENTAGE COMPOSITION OF FLOURS WITH PROTEIN CONTENT AND
DISTRIBUTION OF TOTAL FLOUR PROTEIN IN FRACTIONS

FRACTION		Сомменская		Pawnee			
		F	rotein		Protein		
	Yield a	Percent in Fraction b	Percent of Total Flour Protein in Fraction	Yield *	Percent in Fraction b	Percent of Total Flour Protein in Fraction	
	%	%	%	%	%	%	
Water-solubles	3.6	22.3	10.4	3.7	18.2	6.6	
Gluten	11.2	56.4	81.9	17.6	50.2	87.3	
Tailings	9.8	3.3	4.2	12.3	2.9	3.6	
Prime starch	75.4	0.36	3.5	66.4	0.38	2.5	
Total	100.0		100.0	100.0		100.0	
Percent recovery	96.0			97.5			

Adjusted to 100%; 14% moisture basis.

b 14% moieture basis; factor N × 5.7.

for each flour. Losses were considered as proportionally distributed among all fractions, so the compositions were adjusted to 100% yield and used as the bases for making up treatment combinations required.

Five factors were thus available for interchanging: 1) water-solubles, 2) gluten, 3) starch tailings, 4) prime starch, and 5) composition. Because the relative proportions of components in the two flours differed considerably, it was necessary to include composition as a full-fledged factor, as discussed in the introduction, in order to be able to obtain some measure of the part of the response due to concentration of the other factors. If this composition factor had been omitted, and all the treatment combinations made up at a single composition, the effects of the interchanges would not have been strictly comparable. Perhaps it should be stressed that composition, though a quantity capable of continuous variation, was for this experiment set at two distinct and fixed levels, and was therefore formally equivalent to the qualitative factors comprised by the flour fractions.

The complete set of interchanges was therefore considered as a two-level factorial. Pawnee flour fractions and the composition experimentally found for the Pawnee flour were considered as the Pawnee level, and the commercial fractions and composition were taken as the commercial level. The baking phase of the experiment was arranged as an incomplete block design, set up as a partially confounded 25 factorial. The possible combinations of the five factors called for 32 treatments. Three replicate bakes per treatment (with each replicate the average of two cakes per batter) were deemed sufficient for precision with 16 entries per level of every factor. The design was made up of twelve blocks, each containing eight treatments. Since each block comprised the cakes baked on a single day, some comparisons were partially confounded in order to remove error due to daily variation. It was found that the gain in precision of unconfounded comparisons was negligible, which indicated that day-to-day variation in baking results was not an important source of error.

Reconstituted flours for each combination were prepared by blending the lyophilized components in the proportions required for each treatment, as detailed in Tables IIA and IIB. Each blend was then hydrated to a moisture content of approximately 13%, and bleached with chlorine gas to pH 4.7—4.8. Preliminary tests had demonstrated this to be the optimum bleach level for these materials.

The cake formulation was an adaptation of the Soft Wheat Quality Laboratory's lean white layer formula for varietal evaluation (4). The procedure for use with reconstituted flours and the mixing schedule were as reported previously (2). For each flour combination,

liquid level was adjusted by trial bakes to give the maximum cake volume and best top contour obtainable from that particular flour. It was expected that using the optimum liquid level, as noted in Tables IIA and IIB, for each treatment would eliminate liquid level as a source of variation.

TABLE IIA FRACTION INTERCHANGES, LIQUID LEVELS, AND BAKING RESULTS FOR CAKES USING COMMERCIAL COMPOSITION

TREATMENT	Сом	POSISTION S	PERCENT)	AVERAGE	AVERAGE		
Ne.	Water-Solubles 3.6	Gluten 11.2	Tailings 9.8	P. Starch 75.4	VOLUMB.	Score Score	Lavar p
					ec		%
1	C	C	C	C	604	6.5	103
2	P	C	C	C	576	5.8	103
3	C	P	C	C	500	3.5	97
4	C	C	P	C	596	7.0	103
5	C	C	C	P	613	6.2	103
6	P	P	C	C	449	3.0	103
7	P	C	P	C	516	4.3	109
8	P	C	C	P	583	5.3	103
9	C	P	P	C	458	3.0	103
10	C	P	C	P	496	3.8	103
11	C	C	P	P	580	5.2	103
12	P	P	P	C	442	3.0	97
13	P	C	P	P	541	4.2	103
14	C	P	P	P	489	3.5	103
15	P	P	C	P	493	3.3	97
16	P	P	P	P	438	3.0	103

 a C = fractions from commercial flour; P = fractions from Pawnee flour. b Based on flour weight; flour at 14% M.B.

TABLE IIB FRACTION INTERCHANGES, LIQUID LEVELS, AND BAKING RESULTS FOR CAKES USING PAWNEE COMPOSITION

TREATMENT -	Co	MPOSITION	* (PERCENT	AVERAGE	AVERAGE	Liguin	
No.	Water-Solubles 3.7	Gluten 17.6	Tailings 12.3	P. Starch 66.4	CARE VOLUME	Score Score	Lavar p
					cc		%
17	P	P	P	P	483	3.7	115
18	C	P	P	P	484	3.5	109
19	P	C	P	P	557	6.7	115
20	P	P	C	P	511	3.7	115
21	P	P	P	C	478	4.2	115
22	C	C	P	P	522	5.2	121
23	C	P	C	P	523	5.0	115
24	C	P	P	C	481	3.7	121
25	P	C	C	P	539	6.8	115
26	P	C	P	C	519	5.3	115
27	P	P	C	C	470	3.7	115
28	C	C	C	P	556	6.8	115
29	C	P	C	C	493	4.0	121
30	P	C	C	C	502	5.3	121
31	C	C	P	C	500	4.5	121
32	C	C	C	C	538	6.2	121

^a C= fractions from commercial flour; P = fractions from Pawnee flour.
^b Based on flour weight; flour at 14% M.B.

The primary measure of cake quality was layer volume, as determined by seed displacement. In the absence of an adequate quantitative cake-scoring procedure, the internal score data were not subjected to analysis. These data are reported in Tables IIA and IIB. Obvious differences in cake structures are apparent from the photographs reproduced in Figs. 2, 3, and 4.

Experimental Results

For ease in making comparisons, the baking results are presented in a bar graph, Fig. 1. Here the upper half of the graph represents

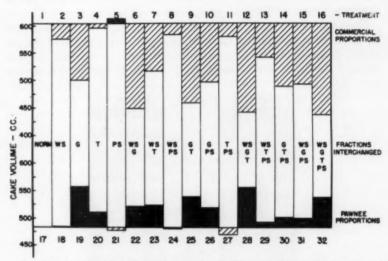


Fig. 1. Variations in volumes of cakes baked with reconstituted flours produced by interchanging four fractions from good- and poor-grade flours. Upper row shows alteration of volumes for flours made up in commercial proportions, and lower row shows changes for those made up in Pawnee proportions. Fractions interchanged are indicated between the rows. Ordinates: layer volume. Lengths of hatched regions 1) in upper row indicate amount of decrease in volume from that of treatment 1 (all commercial fractions in commercial proportions); 2) in lower row, amount of decrease in volume from that of treatment 17 (all Pawnee fractions in Pawnee proportions). The lengths of solid black regions show the volume increase with respect to treatments 1 and 17.

all cakes having commercial flour composition and with commercial flour fractions, except as noted in the center row labeled "factions interchanged." The lower half represents the cakes having Pawnee composition and components, with substitutions of commercial fractions as noted. The ordinate for each half represents layer volume in cc.; the hatched area represents the decrease in volume from that

of the normal (standard) reconstituted cake of each kind, treatment I in upper row and treatment 17 in lower group, and the black area represents the increase in volume over the respective normal reconstituted cakes. In the commercial cakes, substitution of Pawnee gluten was associated in every case with a decrease in volume of no less than 100 cc. Those cakes of commercial composition containing Pawnee water-solubles and tailings with commercial gluten (no. 7, no. 13) had smaller volumes than the normal cake, and those containing Pawnee water-solubles or tailings, or both, with Pawnee gluten, were very poor indeed. Conversely, cakes of Pawnee composition containing commercial gluten were generally larger than the Pawnee control, although volume differences were not so marked.

Figures 2, 3, and 4 show cross-section photographs of representative layers baked from many of the treatments. In addition, the upper row of Fig. 2 shows sections of cakes prepared from the original commercial and Pawnee flours, bleached to pH 4.8 with chlorine. These were

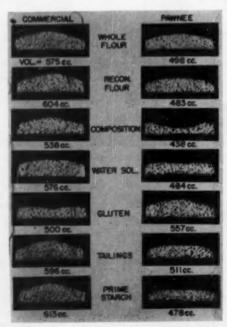


Fig. 2. Rows 1 and 2, comparison of cakes baked from whole and reconstituted flours. Left column commercial, right column Pawnee flour. The reconstituted cakes form treatments 1 and 17 of the treatment combinations. Rows 3 through 7, cakes resulting from single factor interchanges. Factor interchanges noted between the cakes.

obviously of very different baking quality. The second row of cakes in Fig. 2 were prepared from the corresponding normal reconstituted flours: i.e., the commercial reconstituted flour was made up with all commercial fractions combined in the proportions found for that flour (treatment 1), and similarly for the normal reconstituted Pawnee flour (treatment 17). The differences between these reconstituted cakes and their whole-flour counterparts show that, although there was some loss of baking quality attributable to the fractionation, the distinction in quality between commercial and Pawnee was preserved. Consequently, it was felt that whatever damage had occurred to flour factors in fractionation, it was not sufficient to invalidate results obtained from the subsequent interchanges. The other cake sections shown in Fig. 2 were the result of interchanging one factor at a time. For all of these, as well as for Figs. 3 and 4, the column labeled "commercial" comprised layers with all fractions commercial except those interchanged as noted; this was true also for the "Pawnee" column. Interchange of composition (row 3) led to radical decrease of volume in either case, and the layer with Pawnee components was fallen, with very poor internal structure. The "normal reconstituted commercial" cake and the "composition interchanged commercial" cake were both made from all-commercial fractions, i.e., fixed inherent quality, q., for each fraction, but the concentrations, c, in the two cakes were different: this was true also for the two corresponding Pawnee cakes. Here



Fig. 3. Effect of gluten and interaction of other flour components with gluten upon cake volume and structure. Left column, all commercial fractions except for interchanges, and all at the commercial proportions. Right column, all Pawnee fractions except for interchanges, and all in Pawnee proportions.

we have direct evidence that composition, in the sense of relative concentrations, of a flour can have a marked effect on the quality of resultant cakes, apart from any effect attributable to inherent fraction quality. It is clear that commercial composition was superior for the commercial components, and Pawnee composition was superior for the Pawnee components. Notable results of the single-fraction interchanges were: 1) decrease in volume of the commercial cake with Pawnee water-solubles; 2) the inferior quality of commercial cake with Pawnee gluten; and 3) the marked improvement in volume and appearance of Pawnee cake obtained by substitution of commercial gluten.

Figure 3 shows some results of interchanging gluten and other fractions at the same time. In each commercial cake, Pawnee gluten was associated with fallen contour, a remarkable decrease in volume, and very coarse structure. The action of Pawnee water-solubles and tailings, singly, was to decrease the volume and structural quality further, and the cake with all three Pawnee factors was extremely poor. Conversely, the presence of commercial gluten in Pawnee cakes was associated with rounded contour and considerable increase in volume, to such an extent that they appeared similar to the normal reconstituted commercial cakes.

Figure 4 illustrates the effect of prime starch, water-solubles, and tailings in various combinations. In general, these components had much less effect on volume and top contour than did gluten. Presence of the commercial components in Pawnee cakes led to a coarsening of structure. It can be seen, by cross-comparison, that the Pawnee prime

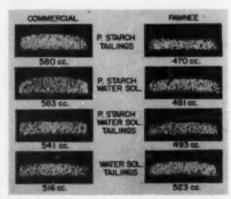


Fig. 4. Effect of prime starch, water-solubles, and tailings interchanges upon cake volume and structure. Left column, all commercial fractions except interchanges, and all at commercial proportions. Right column, all Pawnee fractions except for interchanges, and all at Pawnee proportions.

starch afforded larger volume in both commercial and Pawnee cakes, and that Pawnee water-solubles and tailings together had a greater effect in lowering volume and structural quality of commercial cake than did either component individually.

Analysis

The analysis of variance of the cake volume results is presented in Table III. In the tests of significance, the null hypothesis for each intrafactor comparison was that there was no alteration of volume

TABLE III
ANALYSIS OF VARIANCE OF CARE VOLUME DATA

Source of Variation	d.f.	Mean Squares
Main effects		
Water-solubles	1	10,626
Gluten	1	124,704
Tailings	1	12,331 **
Prime starch	1	7.704
Composition	1	4,428
Interactions		
Water-solubles × comp.	1	2,513*
Gluten × comp.	1	26,800
Tailings × comp.	1	1,980*
W.S. × tailings × comp.	1	4,187
Combined nonsignificant interactions	22	358
Error	53	377

incident upon changing from the Pawnee to the commercial level of a factor. The basic null hypothesis for the interfactor comparisons was that there was no interaction; that is, any change of volume found for the combination of a set of factors was just the sum of the average responses of each. All five factors were highly significant, but the outstanding result of the analysis was the grouping of factors about three centers: 1) effect of prime starch, 2) effect of gluten and its interaction with composition, and 3) effect of water-solubles and tailings and their interactions with compositions.

Cross-comparison of the tests indicates that the responses of the prime starches were significantly different, and that starch was not involved in any significant interaction with other factors. A computed average effect would be a direct measurement of the difference in response to the starches. However, it was quite different with the other factors. The responses to the glutens were evidently quite different at different levels of composition. Since the main effect of gluten and its interaction with composition were both highly significant, and since interaction is essentially the failure of the factors to be additive, an average effect of gluten would not be a direct measure of the

difference in response between the glutens (1). The situation was quite similar for water-solubles and tailings. The responses to combinations of these flour fractions were variable with different flour compositions. Differences in response were great enough that both the main effects and the interactions were significant.

The effect of the composition factor was complex. Variable responses to changes in gluten, water-solubles, and tailings depended upon the relative proportions of the flour fractions. The simplest cause for such variation would appear to reside in the effect of concentration. That is, the effect of flour composition on fraction response as postulated in the introduction may be equivalent to concentration dependence of that response. It should be noted that this type of experimentation with flour deals with a constrained system. Any alteration made to the composition of a flour is restricted by the subsidiary condition that the sum of the percentages of the constituents must equal 100%. Consequently, the effect of concentration on the response of a factor is confounded with the concomitant change in the concentration of at least one other factor. Some results of such variations in flour composition were reported in a previous experiment (2), from which volume response surfaces were derived for the commercial flour. Alterations of a few percent in flour composition resulted in definite differences in cake volume, and it was shown that it is difficult to abstract from experimental results a simple effect of concentration upon the response of any flour fraction.

In the present experiment, two levels were chosen to fix composition, and interchanges of fractions from two flours of very different baking quality were made at these levels, to investigate primarily the effect of fraction quality. However, the concentration dependence of those fractions was also necessarily involved. As used here, the term concentration is intended to mean the effect of composition upon the response of each flour fraction, with due consideration of the complexity of this effect.

Point plots of the responses of each significant grouping are presented in Figs. 5, 6, and 7, to indicate the magnitudes and difference of the effects of each flour factor and to illustrate the extent of interaction when it was present. In these figures the connecting lines are merely visual aids and do not indicate any linear relationship between volume and composition.

Prime Starch. Figure 5 shows the average volumes of cakes baked with the starches at each composition. Each point represents the average volume of 24 cakes. Within the limits of experimental error, the responses to the starches were nearly independent of the composition

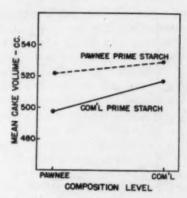


Fig. 5. Effect of prime starch and flour composition upon cake volume.

of the flour, as illustrated by the near parallelism of the connecting lines. The volume change at each concentration was about the same for each starch, averaging 18 cc. in favor of the Pawnee. To that extent Pawnee starch was a better-quality material. Apart from the inherent quality difference, a 9.0% increase in starch concentration (from 66% of Pawnee composition to 75% of commercial composition) effected an increase in volume of about 13 cc. The commercial composition appeared to favor this increase simply because the percentage of starch at this level was higher. The total difference in volume incurred in changing from Pawnee levels of both factors to commercial levels of both was the sum of the responses due to source of starch and concentration, and the net response was negative. It would appear, on the basis of the independence of starch from the other flour fractions, that prime starch quality may be considered as one element in a quality matrix of cake flour. Aside from the question of inherent quality, it appears possible that whatever the role and mode of action of starch may be in the chemistry of baking, it is relatively independent of the quality and amounts of the other flour components, at the levels of concentration studied.

Gluten. Figure 6 is a graph of the average volumes of cakes containing each gluten at each composition. Interaction is evident from the response differences. At Pawnee composition, a change from Pawnee to commercial gluten increased average volume by 38 cc., but at commercial composition, a change from Pawnee to commercial gluten increased average volume by 105 cc. The effect of gluten was undoubtedly the most important item in the entire quality comparison, and it is evident that the degree of response to each gluten was dependent

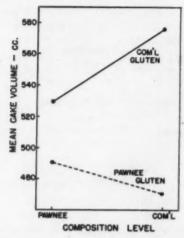


Fig. 6. Effect of interaction of gluten and flour composition upon cake volume.

in some manner upon the amount present. The effect of gluten may be considered from two aspects, 1) layer volume and 2) cake structure. At either concentration, commercial gluten was associated with the better structure and top contour, and the concentration of that gluten affected mainly the volume. But Pawnee gluten was associated with comparable structure only at the Pawnee concentration, and even then layer volume was much smaller. Decrease in concentration led to very poor open crumb and fallen contour, as if the contribution to structural strength had fallen too low. This difference in response between the glutens amounts to a significant inversion, in that the direction of response with respect to concentration for Pawnee gluten was opposite to that of commercial gluten.

We venture to suggest a possible explanation for this inversion, to point up the difference between the glutens and to stress a critical point with respect to the batter-mixing procedure used. Layer specific volume, as a measure of gas-retention capacity and gas phase distribution in crumb, is optimal with small, uniform, thin-walled cells. Any factor tending to produce thick cell walls, holes, or a broad dispersion of cell sizes will impair crumb quality and lower layer volume. For the commercial gluten, granting a basically satisfactory cell formation, the greater response with lower concentration might be associated with decrease in the specific density of gluten in the cellular matrix. That is, with a good-quality gluten, less gluten will do a better job, at least to a point. This corresponds to the practical experience that

low-protein, weak-gluten flours bake the best cakes. The peculiar behavior of the Pawnee gluten can be explained from this point of view only if we conclude that the specific density of this poor-quality gluten is basically far lower than that of the commercial gluten. At higher density (Pawnee composition), it is sufficient to allow the formation of a reasonably good cellular structure, but at lower concentration it fails to provide its contribution simply because there is not enough of it. But there is just as much Pawnee gluten at a given concentration. Hence the key factor, which should resolve this paradox, must not be the amount of gluten present, qua gluten, but the state of the gluten.

It was noted that the commercial gluten peptized readily, so it is possible that one criterion of quality for cake flour gluten is ready solubilization. Hence, ultimate structure formation in layer cake may depend upon the state of the gluten in the batter, more as a binder than as a structural element, in contrast to bread, where gluten does make up the basic framework. The whole formulation and mixing procedure for layer cake batter-neutral to slightly alkaline pH, short mixing time, very high sugar concentration - effect minimum gluten development and afford, perhaps, an environment favorable for solubilization. Because for our reconstituted cake-mixing procedure, a dough step, i.e. gluten development, is preliminary to batter mixing, and the dough is then dispersed by adding sugar, shortening, baking powder, and additional water to form a smooth batter, it seems probable that the ability of the dough to disperse is very critical in the formation of an acceptable layer. By this token, the commercial gluten was sufficiently dispersible that it attained ideal solubilized density at low gluten concentration. By the same token, the Pawnee gluten was inferior because of its greater strength. A stronger gluten should be less dispersible under equivalent conditions; consequently, a greater amount of it would need to be present in order to achieve a sufficient density of solubilized gluten to provide the necessary binding function. A lower concentration of this gluten would not provide sufficient material in the requisite state, so the cellular structure would be poorly developed. In addition, the presence of a large proportion of unsolubilized gluten would be expected to lead to abnormally thick cell walls and to a bready crumb, which was the case with the Pawnee gluten.

Whether such considerations be pertinent or not, on the basis of the structure-volume data presented, it appears that gluten must have had a major role in the formation of the over-all structure of these layer cakes. The problem is, whether gluten made a positive contribution, such as a framework, or as a cementing substance for a starch

structure, in the finished cake, or acted as a limiting agent in the formation of crumb during baking. In these cakes, the combination of weak commercial gluten and low gluten concentration was in the direction of maximizing the quality of this cake structure. The Pawnee gluten and composition did not fit this pattern. Consequently it is inferred that the effect of gluten depends upon both its concentration and its inherent quality in such a way that the quality of the material is the limiting factor. This suggests the possibility of setting up an index to rate various glutens according to the dependence of their response on concentration, much as flours may be ranked on the basis of liquid-level tolerance and optima. Such an index, using a standard set of the other flour fractions, and three or four compositions, would be useful in studies of the quality characteristics of gluten and in the study of the part gluten and gluten protein have in the formation of cake structure. It is hoped that such a study will provide evidence for the hypothesis that solubilization is a factor of importance.

Water-Solubles and Tailings. A third significant set of factors was the response of cake volume to changes in water-solubles and tailings. A graph of the volume changes involving these is shown in Fig. 7. As with the gluten responses, the effect of each component was dependent upon the levels of other factors. For example, at Pawnee com-

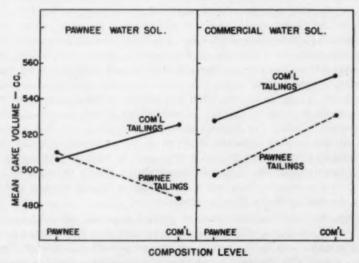


Fig. 7. Effect of interaction of water-solubles, tailings, and composition upon cake volume.

position, change from Pawnee water-solubles and tailings to the commercial components gave an average volume increase of 18 cc., but if the composition were commercial, changing from Pawnee water-solubles and tailings to the commercial components gave an average volume increase of 69 cc. For each of the three factors, the commercial level was in general superior, but the actual volume contribution depended on both the sources of the factors and their relative proportions.

Since the percentage of water-solubles was nearly constant for all treatment combinations, 3.6% in commercial and 3.7% in Pawnee composition, the effect of water-solubles here was not associated with any concentration change. The tailings response, however, was affected by the difference in concentration between the composition levels, amounting to about 20% less tailings for the commercial composition. With commercial water-solubles, tailings response can be attributed to the sum of the effect of tailings concentration and the source, since the response due to the source of tailings was nearly the same at either concentration. With Pawnee water-solubles, the response of commercial tailings was similar to its response with commercial watersolubles, and the lowering of the volume must be a measure of the difference in quality between the two kinds of water-solubles. For these combinations of factors, there was no interaction. But the response of Pawnee tailings with Pawnee water-solubles was opposite to and somewhat lower than its response with commercial water-solubles. This is the interaction shown in the tests of significance.

It will be noted that Pawnee water-solubles, in the presence of commercial tailings, yielded a volume response about 25 cc. lower than did the commercial water-solubles under equivalent conditions. Since Pawnee and commercial composition were about the same with respect to water-solubles, this was a direct measure of the quality difference between the pair. The response attributable to commercial tailings was about 26 cc. greater at the lower tailings concentration afforded by commercial composition, and, under comparable conditions, commercial tailings allowed a 27 cc. greater response than did Pawnee tailings. Consequently, the action of commercial tailings paralleled that of commercial gluten, in that both afforded a greater volume at the lower concentration. With commercial water-solubles. Pawnee tailings had a similar effect; the smaller the amount of tailings. the greater the response, although Pawnee tailings did not allow as much response. The remarkable aspect of this group of factors was the reversal of action of Pawnee tailings when it was used in conjunction with Pawnee water-solubles. The pattern of response involved in this interaction was in the same direction as that of gluten, in that the lowest volume occurred with commercial composition. Figure 3 is a good illustration of the similarity. It is as if a two-factor character of some sort had been separated by the fractionation into a a water-soluble part, W, and a tailings part, T, such that either one had no effect in the presence of its complement, t or w (commercial tailings or water-solubles), or when united in the original proportions WT. But if the amount of T dropped below the original level, as would happen with commercial composition, then the excess of W resulting would act as an inhibitor.

The general effect of water-solubles and tailings appeared to be action on cake structure and volume somewhat similar in type to that of gluten, but not nearly so strong as the gluten effect.

Relative Quality of Flour Fractions and Flour Composition. On the average, over all 32 treatment combinations, the commercial composition was superior to the Pawnee composition. In general, the difficulty in assessing a quantitative measure of the effect of this factor lay in the extent and location of the interactions present, as well as in the confounding of responses due to component quality and component concentration. From analysis of these data it was found that there was negligible interaction of prime-starch response with the other factors. Consequently, it was possible to estimate numerical

TABLE IV
Numerical Estimates of Volume Effect of Flour Fraction

FACTOR	INTERACTION	PERCENT CHANGE IN COMPOSITION (COMM.— PAWNEE)	VOLUME EFFECT * (COMM. — PAWNEE)	Restriction
Prime starch	No		-18** ^{cc}	
Gluten	Large		Not assignable	
Water-solubles	No		+25**	c commercial tailings
	Yes		Not assignable	c Pawnee tailings
Starch tailings	No		+27**	c commercial water-sol.
	Yes		Not assignable	c Pawnee water-sol.
Composition				
a) Starch	No	+9	+13	
b) Gluten	Large	-6.4	Not assignable	
c) Water-				
solubles	No	=0	******	
d) Tailings	SNo.	-2.5	+26**	Excluding Pawnee wa ter-solubles and tail- ings
G) Lainings	Yes	-2.5	Not assignable	With Pawnee water- solubles and tailings

a Significance by t test.

measures of the difference in starch quality and the concentration effect of starch. With each gluten, the response was greatly modified by concentration, although it was certain that the commercial gluten was far superior to the Pawnee component. With water-solubles and tailings, variability of response was attributable to interaction between Pawnee components and concentration. If this combination were omitted, the experimental data allowed an estimate of the superiority of commercial water-solubles over the Pawnee component, of the superiority of commercial tailings over the Pawnee fraction, and of the difference in response due to concentration. These measures are collected in Table IV. This tabulation, made up by analysis of the data presented in Figs. 5, 6, and 7, shows that by averaging out the difference in concentration of fractions between the two compositions, Pawnee prime starch was slightly but significantly superior to commercial prime starch (-18 cc. volume difference); that commercial water-solubles yielded layers 25 cc. larger, on the average, than did Pawnee water-solubles, for those layers not containing Pawnee tailings: and that commercial tailings produced layers averaging 27 cc. larger than did Pawnee tailings, for those layers not containing Pawnee watersolubles.

Further, although the commercial composition contained 9% more starch, on a total percentage difference, than did the Pawnee composition, the increase in layer volume was not significant with respect to starch, but a decrease in tailings concentration by 2.5%, on a total percentage basis (actually about a 20% change in tailings amount), increased layer volume significantly for all layers except those containing both Pawnee water-solubles and gluten. For all other factors and combinations of factors, explicit values could not be obtained because of the various interactions affecting these combinations.

These data appear to support the main contention of the paper, that flour quality might be categorized into groups located in fractions of that flour, and such that each has two aspects, a concentration dependence, c_1 , and an inherent quality aspect, q_1 . Even with regard to the interactions, these are clearly tied in with concentration, so that even though we cannot break them down quantitatively, the pattern of interaction appears to be in accord with the postulate.

Discussion

In undertaking analyses of flours in order to determine how quality factors such as those evaluated herein determine cake quality, several variables must be controlled, particularly with a cake formulation such as the one used for these studies, which is quite sensitive. The

first of these is liquid level, and Kissell has presented data to show the response of cake volume to change in liquid level (4). Although flour response to liquid level is certainly a major quality factor, usually sufficient to mask the expression of many other factors, we felt that it would be better to attempt to eliminate it as a variable for this work. If the liquid level allowing the optimum response of the flour, in terms of volume, top contour, and internal structure, were selected for each treatment combination, we could discount this aspect of the quality of the flours and perhaps obtain valid comparisons of other factors. For these experiments, liquid level for each reconstituted flour was assigned on the basis of trial bakes. The first trial was made using a liquid level of 103% for flours made up using commercial composition, and of 115% for flours made up using Pawnee composition, these levels being the optima of the respective whole flours, and on the assumption that the percentage of gluten was the determining factor as to liquid level required. If a peaked cake resulted, liquid level was cut back 6% (flour basis) and another trial made. If the layer was fallen, liquid level was increased 6%. For each flour, this procedure was followed until layers of rounded contour, which experience has shown is the optimum configuration, were obtained. The liquid level data presented in Tables IIA and IIB show that some treatments did not require adjustment. This procedure was satisfactory for many of the flours; however, it was not possible to obtain rounded contours for some, notably those which contained Pawnee gluten in commercial proportions, which were all fallen to a greater or lesser degree, and for a few others which were peaked or were flat even though the volume was greater than at another liquid level. For these exceptions, the liquid level which yielded the largest volume and best structure was decided upon.

A correlation analysis of the data, liquid level vs. layer volume, gave a coefficient of correlation of -0.11. The fact that there was negligible correlation indicated that the variable was sufficiently well controlled so that it did not constitute a confounding factor. This was particularly important since the cakes containing Pawnee gluten in commercial proportions were all fallen, and the obvious reason for fallen cake is suboptimal liquid level. Consequently it appears that these failures of Pawnee gluten were occasioned by a deficiency of that gluten which became quite marked at the lower concentration. The data indicate, perhaps, that liquid level depended more upon composition than upon any other factor. The Pawnee composition, requiring high gluten concentration, and therefore high protein content, needed the higher liquid levels. A comparison of flour protein,

Tables VA and VB, against liquid level, Tables IIA and IIB, shows that protein content may be the main reason for the higher liquid levels for flours of Pawnee composition. Or the higher liquid level may have been required because of the greater percentage of tailings present at Pawnee composition, since, as shown by Yamazaki (7),

TABLE VA
PROTEIN DISTRIBUTION AND CONTENT IN FLOUR TREATMENT
COMBINATIONS AND CHLORINE DOSAGES.
RECONSTITUTED FLOURS MADE WITH COMMERCIAL COMPOSITION

TREAT-	Pno	CRIN CONTEN	PER FRACTION		F		ΔрН
No.	Water- Solubles	Gluten	Tailings	Prime Starch	PROTEIN	CHIORINE	(ml. Chlorine per g.)
	g/100 g flour	g/100 g flour	g/100 g flour	g/100 g flour	%	ml/g flour	
1	0.80	6.32	0.32	0.27	7.6	0.28	3.0
2	.66	6.32	.32	.27	7.6	.28	2.9
3	.80	5.62	.32	.27	7.0	.28	3.7
4	.80	6.32	.28	.27	7.7	.28	3.3
5	.80	6.32	.32	.29	7.7	.28	3.7
6	.66	5.62	.32	.27	6.9	.28	3.8
7	.66	6.32	.28	.27	7.5	.28	3.9
8	.66	6.32	.32	.29	7.6	.28	3.8
9	.80	5.62	.28	.27	7.0	.28	3.9
10	.80	5.62	.32	.29	7.0	.28	4.0
11	.80	6.32	.28	.29	7.7	.28	4.1
12	.66	5.62	.28	.27	6.8	.28	4.1
13	.66	6.32	.28	.29	7.6	.28	4.3
14	.80	5.62	.28	.29	7.0	.28	4.4
15	.66	5.62	.32	.29	6.9	.28	4.4
16	0.66	5.62	0.28	0.29	6.9	0.32	4.3

TABLE VB
RECONSTITUTED FLOURS MADE UP AT PAWNEE COMPOSITION

TREAT-	I	PROTEIN CONTE	NT PER FRACTION		E. aus	Силовия	ΔРΗ
MENT No.	Water- Solubles	Cluten Tallings Prime PROTEIN	PROTEIN		(ml. Chlorine per g.)		
	g/100 g flour	g/100 g flour	g/100 g flour	g/100 g flour	%	ml.	
17	0.67	8.84	0.36	0.25	10.1	0.35	3.3
18	.83	8.84	.36	.25	10.3	.35	3.4
19	.67	9.93	.36	.25	11.2	.35	2.9
20	.67	8.84	.41	.25	10.2	.36	3.1
21	.67	8.84	.36	.24	10.1	.36	3.1
22	.83	9.93	.36	.25	11.4	.35	3.0
23	.83	8.84	.41	.25	10.3	.35	3.0
24	.83	8.84	.36	.24	10.3	.35	3.6
25	.67	9.93	.41	.25	11.3	.35	2.9
26	.67	9.93	.36	.24	11.2	.35	2.8
27	.67	8.84	.41	.24	10.2	.35	3.2
28	.83	9.93	.41	.25	11.4	.35	2.6
29	.83	8.84	.41	.24	10.3	.35	2.7
30	.67	9.93	.41	.24	11.3	.35	2.7
31	.83	9.93	.36	.24	11.4	.35	2.8
32	0.83	9.93	0.41	0.24	11.4	0.42	2.5

certain tailings components are extremely hydrophylic.

We must remark, however, that the trials with liquid level cannot be considered to be complete. There was insufficient material available to run full-scale (too low to too high) liquid level series. Nevertheless, we feel that the limited work done, with the compromises enumerated, led to sufficiently close approaches to the optima to be satisfactory.

A second variable involved in baking experiments, not only as a variable to be controlled, but as a quality factor in its own right, is the extent of chlorine treatment, since chlorine is a well-known flour improver. It can be seen from the data listed in Tables VA and VB that in general the chlorine dosage required to obtain the desired flour pH, 4.7-4.8, for the treatment combinations was strongly associated with composition and had no correlation with cake volumes and appearance. It is perhaps reasonable that this association with composition would be present. A flour of high protein content, such as all treatments based on Pawnee composition were, presents a buffer system that will require more chlorine per unit pH change than will a low-protein flour. This bears the implication that in chlorine treatment some alteration of the protein, reflected by the pH of the flour, may be a pertinent reason for the improvement. A report in preparation, however, indicates that chlorine dosage and its effect on the physical structure of prime starch are of great importance in flour improvement by this means, and Sollars (6) has presented evidence that a part of the effect of chlorine can be attributed to the starch fraction. However, there is not necessarily a paradox here as far as pH data are concerned. Chlorine treatment of starch increases its acidity, and the liberated hydrogen ion is then free to be taken up by the protein buffer system. Furthermore, it may well be that the action of chlorine is at least twofold.

A third factor requiring evaluation of its effect on flour quality is protein content. As listed in Tables VA and VB, the protein contents of the reconstituted flours ranged from 6.8 to 11.4%. On the face of it, this might be considered sufficient cause for the quality differences found, but an examination of the results leads to just the opposite conclusion.

In this experiment, with flour fractions combined on two concentration bases, there was no way to equalize protein contents of the treatments if, as here, protein contents of the fractions are different, without discarding composition as a factor. One could, of course, make up a number of reconstituted flours from the fractions, all of equal protein content, but there would necessarily be a variety of flour

compositions. A previous report (2) dealt in part with the relation of protein content to cake volume, and it was shown that for several series of compositions, the protein content of those flours presented no clear correlation with cake quality. Here, although we can not equalize protein content without shelving our primary design desideratum, we can control protein distribution closely. We have eight starting materials, four commercial and four Pawnee components, each containing a certain percentage of protein. In the make-up of the treatments, the protein is distributed as shown in Tables VA and VB. For the flours based on commercial composition, all eight treatments using, say, commercial gluten have the same amount of commercial gluten protein, 6.32 g. per 100 g. of reconstituted flour, and all eight treatments using Pawnee gluten have the same amount of Pawnee gluten protein, 5.62 g. per 100 g. flour. For the flours based on the Pawnee composition, eight contain 9.93 g. per 100 g. of commercial gluten protein, and eight contain 8.84 g. per 100 g. of Pawnee gluten protein; the data are analogous for all the other fractions in appropriate combinations. The net result is 32 flours with nearly as many protein contents, grouped roughly as high-protein (Pawnee composition) and lowprotein (commercial composition).

But note that in the additions and subtractions for the estimates of the volume responses, these fraction protein contents segregate with their flour fractions. Hence, the estimates of response to flour fractions are entirely equivalent to the estimates of response to protein content, so volume response and protein content of each factor can be compared directly.

Consider the gluten × composition interaction factor pairs of Table VI. The average increase in volume with the commercial gluten when commercial gluten protein is decreased from 9.93 g. per 100 g. flour to 6.32 g. per 100 g. is 47 cc., which certainly indicates that a lower gluten protein content is better. But the average volume change for decreasing Pawnee gluten protein is a decrease of 20 cc. The table shows that the highest gluten protein content is associated with an intermediate average volume, but the lowest gluten protein content is associated with the lowest average volume. In short, there is no apparent relation between protein content per se and the volume response.

The relation shown for gluten × composition interaction is that the responses due to commercial and Pawnee gluten are opposite. Although the factors responsible are located in the gluten, whether the factors are in the protein part or the nonprotein part of the gluten is at this time impossible to say. To locate them precisely would require

TABLE VI
COMPARISON OF FLOUR FRACTION PROTEIN CONTENT WITH AVERAGE CAKE VOLUME

Factor(s)		Dansen Courses	AVERAGE VOLUME	
Fraction(s)	Composition	PROTEIN CONTENT		
		g/100 g flour	cc	
Pawnee gluten	Commercial	5.62	471	
Pawnee gluten	Pawnee	8.84	491	
Commercial gluten	Pawnee	9.93	529	
Commercial gluten	Commercial	6.32	576	
Commercial starch	Pawnee	0.24	498	
Commercial starch	Commercial	0.27	518	
Pawnee starch	Pawnee	0.25	522	
Pawnee starch	Commercial	0.29	529	
Commercial water-solubles Commercial tailings	Commercial	1.12	553	
Commercial water-solubles Commercial tailings	Pawnee	1.24	528	
Commercial water-solubles Pawnee tailings	Commercial	1.08	531	
Commercial water-solubles Pawnee tailings	Pawnee	1.19	497	
Pawnee water-solubles Commercial tailings	Commercial	0.98	525	
Pawnee water-solubles Commercial tailings	Pawnee	1.08	506	
Pawnee water-solubles Pawnee tailings	Commercial	0.94	484	
Pawnee water-solubles Pawnee tailings	Pawnee	1.03	509	

fractionation of the gluten or chemical analysis, and to explain their action would require detailing of a mechanism of gluten action in cake crumb formation. But the point here is that explanation of response on basis of protein determination is inadequate.

This inference is reinforced by evaluation of the water-solubles × tailings × composition interaction. Table VI shows that there is no clear dependence of average volume on protein content. The lowest protein content of the combination of fractions is associated with the lowest volume response, but an intermediate protein level is associated with the greatest volume response. It is of interest to note that if the Pawnee water-solubles and tailings interaction be neglected, and the protein contents of the fractions computed for each combination, then decrease in tailings protein gives larger volume, but decrease in water-solubles protein is associated with smaller volume.

These considerations, taken together with the lack of correlation of flour protein content with cake volume, can only indicate that protein content is not an adequate explanation of the quality differences found. In particular, although protein content was not controllable and so varied from one flour to another, protein distribution was controlled in the design. Hence results were not invalidated by the inequalities of protein content. In fact, the results indicate rather pointedly that protein content can be a poor basis for measuring

quality.

It will be noted from the data in Table I that 82-87% of the flour protein of each flour fractionated was found in the gluten, although the protein contents of the glutens were 50-56%. It seemed to us that the best method of fractionation for quality testing by baking experiments would be one which made the least possible modification to the flour components, commensurate with providing, say, four cuts qualitatively and analytically distinguishable. It was considered that a process using distilled water as the sole medium, as small an amount of manipulation of the material as possible, the least loss possible, and batch-to-batch reproducibility of relative proportions and protein analysis of each fraction, was requisite. The batter process developed fulfills these criteria better than any other procedure we have explored. Admittedly, each fraction probably contained a small amount of occluded material properly belonging to other cuts. Except for gluten, which was washed three times in distilled water, and the small refinement detailed above in the "Materials and Methods" section, the fractions were freeze-dried without any further treatment. Qualitatively, the water-solubles obtained were, after drying, very feathery and hygroscopic. The prime starches were low in protein, the tailings cut was quite fibrous and fluffy, and the gluten appeared quite homogeneous and free from prime starch. The boundary between tailings and prime starch, after centrifugation, was well defined.

Although it would certainly be possible to obtain, for example, gluten of much higher protein content, the necessary additional kneading with salt additions (the commercial gluten began to show distressing signs of peptizing after several washings in distilled water) or dispersal in acid and reprecipitation makes it problematical that the gluten would not be further changed by such treatment beyond the development necessary to make the initial separation. Since state of purity of complex mixtures, such as flour fractions are, is a rather arbitrary concept, once one begins to purify, there is always the problem of how to class the new cuts obtained by the refining process. After all, the sort of gluten that one obtains from a fractionation process depends to a considerable extent on how one isolates it. In short, "gluten" is very much an operational definition. There is, to our knowledge, no prior reason, and very few experimental reasons, why gluten should not be half protein and half nonprotein in com-

position, at least as far as cake-baking is concerned. The distressing similarities of gluten protein amino acid analysis as found in the literature (e.g. 5), may well indicate that the characterization of gluten as a high-protein material is far too restrictive to be useful in evaluating gluten quality in baked goods produced from soft wheats. Indeed, gluten solubilization characteristics may well be the best single criterion of gluten quality. The superiority of the commercial gluten, shown herewith, can certainly be interpreted as evidence for this point of view, although any rational basis for decision as to what constitutes quality must await elucidation of baking mechanisms in terms of chemical or physical processes.

The fact that one can bake from many of these synthetic flours a cake that has volume and appearance similar to the cake made from the original flour, using an extremely lean and consequently very sensitive cake formula, indicates that the fractionation procedure has not altered the starting material too greatly.

We should like to make explicit a methodological assumption implicit in this sort of experimentation. In evaluating data from reconstitution bakes, one is faced with two problems, aside from the questions regarding loss of quality entailed by fractionation itself: 1) evaluating the role of the flour factor or component in the intact flour, since very little is known of the mechanisms of cake baking, and 2) evaluating the action of the component in a reconstituted flour, where even mere physical separation may have altered the function of the component. This is particularly difficult with respect to gluten, since isolation of the material changes its physical state materially. But, if one obtains a layer from a reconstituted flour that is reasonably similar to one obtained from the intact flour, he must suppose that whatever the operations performed on the two flours, the end results are occasioned by the same mechanism, so that the state and function of the components are similar in the cakes produced from both the intact and the reconstituted flours, until proved otherwise. The alternative to operating in this way is to discard the analytical approach and try a holistic one, which seems to us to be far less promising.

Conclusions

It may be inferred from the analysis of the data that, with respect to commercial tailings and gluten, the commercial composition, which gave lower concentrations of both factors, was very much superior to the Pawnee composition, and that regarding inherent quality, the commercial gluten and tailings were superior to the Pawnee fractions. With respect to water-solubles, the commercial kind was definitely of superior quality. With respect to starch concentration, a larger quantity was slightly superior, at least in the range of compositions used; and with respect to starch quality, the Pawnee component was slightly better. The marked decrease in cake volume and deterioration of cake structure when Pawnee gluten, water-solubles, and tailings were used in commercial proportions indicated that the inferiority of such components was aggravated by decreasing their concentration, as if the structural strength had fallen below a critical value. The effect of Pawnee tailings was so different with Pawnee and commercial water-solubles that it was inferred that some kind of reinforcement occurs between the Pawnee components, and that this supplements the effect of Pawnee gluten.

The work suggests that fraction responses might be divided into three groups, and that a relative rank can be assigned to each. This division comprises a small prime starch contribution, a very large gluten contribution, and a moderate tailings and water-solubles contribution to cake quality. The analysis indicates that the prime starch contribution is essentially independent of the other two. The gluten, water-solubles, and tailings contributions were related by the presence of concentration effects which were somewhat similar. The implication is rather strong that the gluten, water-solubles, and tailings determine the quality of a basic matrix in the cake structure. A good-quality starch leads to a better cake than a poor-quality starch, other things being equal, but if the basic matrix is of poor quality, it overwhelms the contribution of the starch.

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EFFECT OF BISULFITE AND ACETALDEHYDE ON THE DISULFIDE LINKAGE IN WHEAT PROTEIN¹

H. MATSUMOTO, I. OSHIMA, AND I. HLYNKA

ABSTRACT

Wheat proteins extracted from flour with N/100 acetic acid following a preliminary extraction with water were treated with bisulfite. The increase in sulfhydryl content was followed titrimetrically, using the mercury pool electrode. Only a small increase in -SH was found after 3-minute reaction time. A rapid increase in -SH followed in the next 2 or 3 hours. Urea increased the rate at which equilibrium was attained. With the addition of acetaldehyde a rapid decrease in -SH content was observed at pH 6 and 7 and a slower decrease at pH 4 and 5. N-ethylmaleimide and bromate decreased the titration value prominently. The equilibrium constant for the reaction of bisulfite with wheat proteins was obtained. The above chemical effects are supported by the physical effects of bisulfite and acetaldehyde on the mixing characteristics of dough in the farinograph.

A study of the disulfide-sulfhydryl system of wheat proteins should contribute significantly to a better understanding of the fundamental structure and properties of dough. One approach to this problem has been to focus the attention primarily on the sulfhydryl group as was done by Kong, Mecham, and Pence (9), Bloksma (2), and by Matsumoto and Hlynka (13). Another approach is to place emphasis on the disulfide cross-linkage. This second approach has been adopted in the present investigation. Bisulfite was selected as a reagent that has a prominent effect on the disulfide linkage, and the reaction was followed titrimetrically with the newly developed mercury pool electrode (8).

The effect of bisulfite on the mixing properties of dough was shown by Freilich and Frey (3) in a comparative study of oxidizing and reducing agents. Merritt and Bailey (11) reported that the addi-

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tion of bisulfite resulted in an increased extensibility and a decreased resistance to extension of doughs tested with the extensigraph. Using both the extensigraph and the farinograph, Hlynka (5) studied the effect of bisulfite and acetaldehyde and interpreted his results in terms of the cleavage of a cross-linked network structure of dough.

Additional information was provided by Olcott, Sapirstein, and Blish (14) and Udy (20), who followed the changes in viscosity of gluten dispersions in dilute acetic acid, treated with small amounts of bisulfite. The former authors concluded that the direct reaction of bisulfite with gluten, in decreasing the relative viscosity, was a primary one, and by implication that the activation of proteinase was a minor consideration. Udy suggested a possible fragmentation resulting from the cleavage of the disulfide linkage. He also showed that bisulfite seemed to have some effect on the control of electrostatic forces between protein molecules.

Matsumoto (12) reported a comparative study on the viscosity change and on the sulfhydryl content in gluten determined by amperometric titration (1), using a rotating platinum electrode in ammoniacal buffer and urea solution. He showed that the increased titration value with bisulfite came from the cleavage of the disulfide to sulfhydryl in gluten. The amperometric titration with the rotating mercury pool electrode made possible the titration at a lower pH nearer to that of normal dough. In the present investigation the mercury pool electrode was used, with the modification that the electrolyte was stirred with a magnetic stirrer. The results obtained in the study of the effect of bisulfite and acetaldehyde are reported.

Materials and Methods

The flour used in this study was an unbleached, improver-free, straight-grade flour commercially milled from Manitoba No. 2 Northern wheat. The protein content of the flour was 12.6%. The gluten dispersion was prepared according to the procedure described in our previous paper (13), with details as follows:

The flour was extracted with water at first, with a water: flour ratio of 7:1. The gluten dispersion was obtained by extracting the residue from the water extraction with N/100 acetic acid, with the acid solution: original flour ratio of 4:1. This dispersion contained 1.42 to 1.60% protein and had a pH of 5.0. All of the reactions and manipulations reported in this paper were carried on in a nitrogen atmosphere.

Amperometric Titration. The mercury pool electrode, described by Kolthoff, Anastasi, and Tan (8), was used. It was a glass cup about 5 mm. in height and 6 mm. in diameter, filled with mercury. The electrolyte was mixed with a magnetic stirrer at 100 r.p.m. instead of rotating the electrode.

The composition of electrolyte (final concentration) was:

Gluten (dispersed	in N/100	acetic acid)	0.71-0.7	77%
Urea			6.7M	
KCl				

The potential of the electrode vs. a saturated calomel electrode was -0.15 volt. The titration was carried out with M/500 mercuric chloride at room temperature. For these titrations the pH of the electrolyte was not adjusted in the range of pH 5 to 2; the titration values obtained in this range were within a standard deviation of 2.4%. When the pH was higher than 5, the solution was adjusted to pH 5 with hydrochloric acid. End-points were determined from the current-titration curves. Calculation was made to correspond to the ratio 1 HgCl₂ to 2 SH. The recovery of glutathione added to the above electrolyte was 118% by this method.

Farinograph Tests. Dough was mixed from 50 g. flour with 50 p.p.m. bisulfite for 3 minutes in a small farinograph bowl with the normal absorption decreased by 10 ml. Then it was rested for 5 minutes at 30°C. as reaction time. After a rest period, 250 p.p.m. acetaldehyde were incorporated into the dough with 10 ml. of water and the dough was mixed again to obtain a farinogram. In the control experiment 10 ml. of water were added instead of the reagent. pH was adjusted with phosphate buffer.

Results

The results are presented in five sections. Data on the effects of bisulfite and its dependence on reaction time are summarized first. Then follow observations on the effect on the bisulfite reaction of pH, acetaldehyde, oxidizing agents, and specific sulfhydryl reagents. Finally farinogram tests are shown.

1. Effect of Bisulfite and Its Reaction Time on Sulfhydryl Content of Gluten Dispersion. Solid bisulfite was added to gluten dispersions. The reaction was carried out under an atmosphere of nitrogen at 30°C. The final concentration of urea in the titration vessel was adjusted to the same level as shown above.

The results are shown in Fig. 1. Both with and without the addition of urea, only a small increase in sulfhydryl was found after 3 minutes' reaction time. An increase in sulfhydryl content followed during the next 2 or 3 hours, but only with solutions containing urea

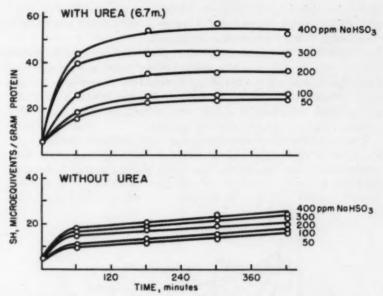


Fig. 1. Sulfhydryl increment versus reaction time on the reaction of gluten dispersion with bisulfite at pH 5.

was equilibrium finally attained in this time. The reaction was faster with urea, as indicated by a higher sulfhydryl content found.

2. Effect of pH on the Reaction of Bisulfite. Gluten dispersions without urea cannot be adjusted to the desired pH, since precipitation results. They were therefore treated with 200 p.p.m. bisulfite in 6.7 molar urea at various pH's, adjusted with sodium hydroxide or acetic acid.

The results are shown in Table I. No large difference in the sulf-

TABLE I

PH	-SH (Microequiv. per g. Protein)	
	After treatment	
2.0	10.8	
3.0	26.8	
4.0	38.0	
5.0	37.2	
6.0	37.6	
	Before treatment	
5.0	3.0	

a 3 hours' treatment with 200 p.p.m. bisulfite.

hydryl increment in the range of pH 4 to 6 was found. The small sulfhydryl increment at pH 2 to 3 seems to show a low reactivity of the disulfide linkage under these conditions.

3. Effect of Acetaldehyde on Sulfhydryl Content of Gluten Dispersion Treated with Bisulfite. After 3 hours' reaction time with 200 p.p.m. bisulfite at pH 5, 440 p.p.m. of acetaldehyde were added to the reaction mixture of a gluten dispersion adjusted to the desired pH. The first and the second reactions were carried out in 6.7 molar urea solution dissolved directly in the dispersion.

The results of the determination of free sulfhydryl are shown in Fig. 2. With the addition of acetaldehyde, a rapid decrease in sulfhydryl content was observed at pH 6 and 7 and a slower decrease at pH 5 and 4.

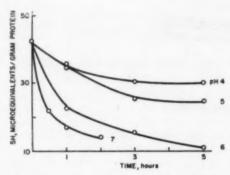


Fig. 2. Effect of acetaldehyde on sulfhydryl groups of gluten dispersion treated th bisulfite at various pH levels.

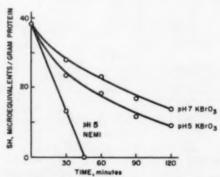


Fig. 3. Effect of bromate or N-ethylmaleimide on sulfhydryl groups of gluten dispersion treated with bisulfite.

4. Effect of NEMI or Bromate on Sulfhydryl Content of Gluten Dispersion Treated with Bisulfite. After 3 hours' reaction time with 200 p.p.m. of bisulfite, 400 p.p.m. of N-ethylmaleimide (NEMI), a specific reagent for the sulfhydryl group, or 200 p.p.m. of potassium bromate, were added to the mixture.

The results of the determination of free sulfhydryl groups at various times after the addition of the last reagent are shown in Fig. 3. The dough containing NEMI had a pH of 5 and the doughs with bromate were at pH 5 and pH 7.

5. Effects of Bisulfite and Acetaldehyde on Farinograms at Different pH. Bisulfite and acetaldehyde were incorporated into the dough at the same time by Hlynka (5). In this experiment, acetaldehyde was mixed into the dough 8 minutes after the bisulfite had been incorporated initially into the dough at pH 5 or 7. Results are shown in Fig. 4.

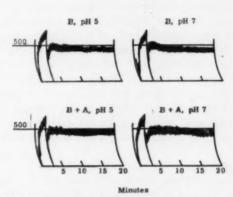


Fig. 4. Effect of bisulfite and acetaldehyde on farinograms at pH 5 and 7. B, Bisulfite, 50 p.p.m.; A, Acetaldehyde, 250 p.p.m. Initial mixing 3 minutes with bisulfite, with absorption decreased by 10 ml. Rest period 5 minutes. Final mixing 17 minutes with or without acetaldehyde in 10 ml. water.

These farinograms show that the addition of acetaldehyde, after the reaction of dough with bisulfite, had little effect at pH 5. At pH 7 a definite increase in consistency is shown as a result of treatment with acetaldehyde.

Acetaldehyde without bisulfite was confirmed to have no effect on the farinogram at both pH 5 and 7. However, it is not shown in Fig. 4, as the result is the same as reported by Hlynka (5).

Discussion

Some studies on the effect of bisulfite on the disulfide linkage of

cystine, oxidized glutathione, and protein (16,17) bovine serum albumin (8), and wool (4,18) have been published recently. In these studies the following mechanism of the reaction is generally accepted.

$$\begin{array}{c|c}
S \\
P \\
+ SO_3 = + H^+ \rightleftharpoons P \\
S.SO_3^-
\end{array} (1)$$

where the protein disulfide is denoted by $P \begin{vmatrix} S \\ S \end{vmatrix}$.

It is reasonable to assume the same type of reaction for gluten and bisulfite. This reaction of bisulfite with gluten, breaking the disulfide linkages of protein into sulfhydryl and sulfonate groups, has been confirmed experimentally in the present study through amperometric titration of the sulfhydryl group produced.

However, some comment should be made on the effect of bisulfite on this titration itself. The condition of this titration is quite the same as Kolthoff's (8), except that urea is used as a denaturing agent instead of guanidine hydrochloride, and the sample is gluten dispersion in acetic acid. The urea and acetic acid were found to have no inhibiting effect on this titration in blank tests with them. On the basis of this observation and the fact that N-ethylmaleimide (which is a specific reagent for the sulfhydryl group) can cancel the titration value, the increased titration value is presumed to come from sulfhydryl groups of gluten.

The effect of bromate in markedly decreasing the titration value should be considered from two aspects. One is a reaction of bromate with sulfite which causes shifting the equation (1) to the left, and the other is the direct oxidation of sulfhydryl groups of gluten. The effect of pH on the oxidation seems to support the latter possibility. The question arises whether the sulfhydryl is produced during the reaction, or results from the treatment of the titration electrolyte with urea. The results obtained for the 3 minutes' reaction time in Fig. 1 indicate very little increase of titration value. Thus the increased titration value may be considered as a result of reaction time and not as that of treatment during the titration. This is also known from titration curves which show no decrease in current with each addition of mercuric chloride solution. These curves, which served mainly as primary data, are not shown in this paper.

The data shown in Fig. 1 indicate that urea increases the velocity of attaining equilibrium and also produces more sulfhydryl groups

than is produced in the reaction without urea. It is reasonable to assume that the breaking of hydrogen bonds by urea may increase the susceptibility of protein toward bisulfite.

An equilibrium constant for equation (1) can be calculated from titration values with the assumption that all disulfide groups in gluten are reactive and have the same equilibrium constant, and the reactions are consecutive.

The constant is shown by the equation (2) at a given pH.

$$K = \frac{[-SH] [-S.SO_3^-]}{[P(S-S)] [SO_3^-]}$$
 (2)

The values for each bisulfite concentration in Fig. 1 are summarized in Table II.

TABLE II
EQUILIBRIUM CONSTANT IN EQUATION (2) AT 30°C. WITH OR WITHOUT 6.7M
UREA AT PH 5.0

BISULPITE		DIN &	K			
РРЖ P	PSH	PSSO ₀ -	P(S-S)	SO 8 "	Α.	
	× 10-4	× 10-4	× 10-4	× 10 ⁻⁴		
With urea						
50	3.7	3.0	10.0	0.59	1.88	
100	4.2	3.5	9.5	2.01	0.77	
200	5.8	5.1	7.9	4.65	0.81	
300	7.1	6.4	6.6	7.39	0.93	
400	9.1	8.4	4.6	10.00	1.66	
Blank	0.7	0	13.0			
Without urea						
300	3.2	2.5	9.3	8.80	0.09	

a After equilibrium was attained.

In these calculations, SO₃= was obtained from bisulfite added to the solution through the following equations:

$$HSO_3^- \rightleftharpoons H^+ + SO_3^=$$
 (3)
 $H_2SO_3 \rightleftharpoons H^+ + HSO_3^-$ (4)

Denoting by a the expression,

$$\frac{SO_3^{=}}{H_2SO_3 + HSO_3^{-} + SO_3^{=}}$$
then $\frac{1}{a} = 1 + \frac{1}{K_2} [H^{+}] + \frac{1}{K_1K_2} [H^{+}]^2$ (5)

where K_1 and K_2 are the equilibrium constants of equations (4) and (3), and were taken as 1.7×10^{-2} and 5×10^{-6} respectively (10). The calculated α was 0.33 at pH 5. P (S-S) in the table was obtained from the total cystine content and the reacted disulfide. For the calibration the cystine content was assumed to be 2% of the total gluten. This

value was based on published literature (13,15) and was also checked experimentally.

Some of the equilibrium constants at each bisulfite concentration were closely reproducible, while some indicated higher values. These higher values may be explained with the assumption that the sulf-hydryl groups that come into solution at lowest and highest bisulfite concentrations have a different reactivity. The K value in the reaction without urea is about one-eighth of that with urea. This reactive disulfide, calculated from the data obtained in the reaction with bisulfite at 400 p.p.m. in the reaction mixture which produced maximum sulfhydryl content in Fig. 1, was 63% of total disulfide calculated from the cystine content.

Udy (20) showed that the intrinsic viscosity of gluten dispersions from different flours which originally had different values reached the same value after the reaction with bisulfite. The differences in intrinsic viscosity of gluten dispersions may be related to the reactive disulfide, because both the differences between samples in intrinsic viscosity and in reactive disulfide linkages disappear after the reaction with bisulfite.

The results in Fig. 2 show very good agreement with those reported by Hlynka (5) in studies of the physical properties of dough and gluten. The reaction of acetaldehyde which decreased the sulfhydryl groups produced by bisulfite shows strong evidence of its counteraction to bisulfite, although this is very slow except at pH 6 and 7.

This reverse reaction may be explained by the following possible reactions:

(a) Reverse Reaction of Equation (1). In the addition of acetaldehyde to the reaction mixture, bisulfite is removed from the reacting system by forming an addition compound with acetaldehyde which shows very little dissociation at pH lower than 8 (19). The equation (1) is shifted to the left, assuming it to be reversible. As a result, the titratable sulfhydryl groups are decreased.

(b) Mercaptal Formation. Acetaldehyde may react with sulfhydryl groups of gluten produced by the reaction with sulfite, to form a mercaptal at high pH level which prevents the sulfhydryl groups from titration with mercuric ions.

(c) Oxidation of Sulfhydryl Group. A small amount of oxygen may be left in the reaction mixture, and may oxidize sulfhydryl groups at a higher pH level in the medium where excess bisulfite was eliminated from the reaction mixture.

This third possibility can be neglected, because in this study acetaldehyde was added to the mixture which was freed from oxygen

by twice-repeated evacuations and filling with nitrogen alternately.

The first possibility depends on the reversibility of equation (1). The authors checked this reversibility by breaking the equilibrium of the equation through vacuum evaporation of sulfur dioxide at a low pH level. At pH 2.8 the titration value of the reaction mixture of Section 1 was approximately the same before and after evaporation of sulfur dioxide. However, the mixture raised to pH 6 after the evaporation at pH 2.8 decreased in sulfhydryl content to about onehalf. Stricks and Kolthoff (16) described the reversibility of the cysteine-cystine system in the presence of sulfite in the pH range from 8 to 13. Thus the first mechanism appears to be more probable.

However, the alternate possibility of mercaptal formation cannot be neglected.

The farinograms shown in Fig. 4 also strongly support these results. That is, a definite increase of consistency is observed on the addition of acetaldehyde at pH 7 but not at pH 5, where little decrease of sulfhydryl groups is detected in Section 3. The free sulfhydryl groups in water-soluble and acid-soluble proteins were estimated before and after oxidation by Matsumoto and Hlynka (13). The value of free sulfhydryl was very small compared with disulfide linkage. Thus the sulfhydryl-disulfide exchange reaction is suggested as part of this system too (6,7). The reactive disulfide in gluten studied in this paper also seems to play an important part in the sulfhydryldisulfide exchange reaction. Further work on this disulfide linkage may eventually build up a bridge between rheological behavior and basic structure of dough.

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MEASUREMENT OF THE IMPROVER RESPONSE IN DOUGH1

I. HLYNKA AND R. R. MATSUO

ABSTRACT

Structural relaxation data were obtained for doughs containing initially 0, 10, 15, and 20 p.p.m. bromate and allowed reaction times of 1, 2, and 3 hours. When the semiaxis constant for the bromate effect was plotted against reaction time, linear plots were obtained. Analogous linear plots were also obtained when the amount of bromate reacted in dough as determined chemically was plotted against reaction time. The amount of change in the semiaxis constant per unit amount of bromate reacted was taken as a definition of bromate response. The response was evaluated for several flours. Improver response was also obtained from similar data with iodate.

When bromate or iodate reacts with dough a change in the physical properties of the dough results. If our knowledge of dough chemistry and rheology were adequate, it should be possible to predict from the bromate treatment the corresponding change in the physical properties of dough, and conversely.

There have been two attempts made to evaluate the bromate response of dough from physical measurements. Munz and Brabender (6) derived an "oxy-number" based on the area under the extensigram, and the ratio of its height to length. Merritt and Bailey (5) also included the protein content of flour as a factor in a similar expression to obtain an "age index."

In a recent paper (3) the writers presented preliminary evidence suggesting that the semiaxis constant of the structural relaxation curve offered certain advantages as a measure of the bromate response in dough; a simple linear relation was obtained between this rheological parameter and the initial concentration of the bromate added to dough. The present paper is an extension of this study. On the one hand, rheological data in terms of the semiaxis constant were obtained for a series of doughs at several levels of bromate, and after different reaction times. On the other hand, parallel chemical determinations were made on similar doughs and the amount of bromate reacted after different reaction times was obtained. Supplementary data were also obtained with iodate. These data are presented and discussed in terms of the physical response of the dough in relation to the amount of improver reacted.

¹ Manuscript received March 17, 1960. Paper No. 188 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg 2, Manitoba.

Materials and Methods

Four flours designated A, B, C, and D, all commercially milled from Canadian hard red spring wheat, were used for this study. Flours A and B were straight grade; flour C, termed "baker's special," was the first 70% and flour D, termed "baker's strong," was the remaining 30% of the total yield of flour. All flours were unbleached and improver-free. The particulars are listed in Table I.

TABLE I DESCRIPTION OF FLOURS

		FLOUR				
	A	B	С	D		
Protein content, %	12.2	13.2	12.2	14.9		
Ash content, %	0.47	0.46	0.34	0.66		
Farinograph absorption ^a	59.6	64.5	62.3	66 7		
Optimum bromate requirement, p.p.m.b	5.0	10.0	5.0	17.5		

a A uniform absorption of 55% (14% basis) was used for all flours.
b AACC baking test.

A uniform absorption of 55% (14% flour basis) was used for all ours so as to keep the total water-to-dry-flour ratio constant and in his way to make comparisons of the different flours on an equal basis.

Doughs were prepared by mixing 200 g. of flour (14% moisture pasis), salt solution, and varying amounts of bromate or iodate solution in an atmosphere of nitrogen for 2.5 minutes in a GRL mixer (2). Temperature was controlled so that the dough came out of the mixer at 30°C., and it was maintained at this temperature in a humidified cabinet during reaction time and rest period.

Structural relaxation curves were obtained for doughs containing 0, 10, 15, and 20 p.p.m. potassium bromate and allowed reaction times of 1, 2, and 3 hours, and for doughs containing 0, 5, 10, and 15 p.p.m. iodate and allowed reaction time of 1 hour. The methods used in obtaining the curves, in evaluating the relaxation constant C and the semiaxis constant a were those described in the previous study (3).

In the present study a simple linear relation was found between the semiaxis constant and reaction time by the following modification. The relaxation curve for the control dough was subtracted from the curve for the bromated or iodated dough to give a new curve which represented the rheological change due to the action of the improver. The semiaxis constant for the new curve was then obtained. In practice this was evaluated from the relation

$$a = \sqrt{2} \left(C_{\text{bromated}} - C_{\text{control}} \right)$$

where the symbols C and a have the usual meaning. The procedure for doughs treated with iodate was the same.

A set of bromate-treated doughs entirely similar to those used for the extensigraph tests was used to obtain parallel chemical data. The bromate loss in dough for each bromate concentration and reaction time was determined by the method of Cunningham and Anderson (1), and the results were plotted as the amount of bromate reacted against given reaction time.

Results

Variation of the Semiaxis Constant a with Reaction Time. The first set of experiments was designed to provide structural relaxation data for doughs containing initially 0, 10, 15, and 20 p.p.m. bromate and allowed reaction times of 1, 2, and 3 hours. Data on four different flours were obtained.

Figure 1 summarizes the rheological data. The semiaxis constant a for the bromate effect is plotted against reaction time for three bro-

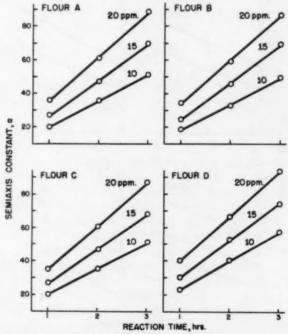


Fig. 1. Plots of the semiaxis constant a against reaction times for doughs initially containing 10, 15, and 20 p.p.m. bromate.

mate levels. The linearity of the plots is immediately apparent for all four flours. This contrasts with the curvilinear plots obtained by the method described in the previous paper (3). The data in the present form have the important advantage that they can be compared with analogous chemical data to be presented in the next section.

Although the flours used in this study differed appreciably, the bromate effect as measured by a was very nearly the same. This similarity in the physical response of the flours to bromate is better

TABLE II
CHANGE OF THE SEMIAXIS CONSTANT & WITH REACTION TIME OR SLOPE

	SLOPE							
INITIAL BROMATE	Flour A	Flour B	Flour C	Flour D				
ppm								
10	15.6	15.3	15.6	17.2				
15	21.0	22.1	20.5	21.9				
20	26.3	26.2	25.9	26.6				

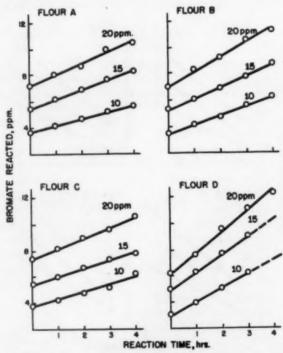


Fig. 2. Plots of the amount of bromate reacted in dough against reaction time for doughs initially containing 10, 15, and 20 p.p.m. bromate.

shown by the slopes of the a vs. reaction time plots summarized in Table II.

Variation of the Amount of Bromate Reacted in Dough with Time. Parallel with the rheological study, doughs for the chemical analysis of bromate were prepared in the same manner with the same initial bromate concentrations and given reaction times of 0, 1, 2, 3, and 4 hours. Figure 2 summarizes the results. The amount of bromate reacted is plotted against reaction time.

The linearity of these plots is in accordance with the previous finding that the rate of loss of bromate is constant for a given initial concentration (4,1). The rates of bromate loss for each of the flours obtained as the slope of the individual graphs are summarized in Table III.

TABLE III
RATES OF BROMATE LOSS IN DOUGH

INITIAL BROMATE	RATE OF LOSS									
	Flour A	Flour B	Flour C	Flour D						
ppm	ppm/hr	ppm/hr	ppm/hr	ppm/hr						
10	0.51	0.65	0.50	1.08						
15	0.71	0.84	0.65	1.38						
20	0.87	1.11	0.81	1.64						

Variation of Semiaxis Constant a with Bromate Concentration c. There is a striking similarity between Figs. 1 and 2 in that the rheological and chemical data, both plotted against reaction time, are linear. This similarity makes it possible to study these data further.

The amount of change in the rheological parameter per unit amount of bromate reacted may be looked upon as a measure of bromate response (a/c) of the dough. Figure 3 (left) presents a plot of the semiaxis constant a against the amount of bromate reacted c. The initial amount of bromate in the dough was 20 p.p.m. and the data plotted were obtained after reaction times of 1, 2, and 3 hours. Other initial levels of bromate gave essentially similar results and are not shown.

The response (a/c) of each of the flours to bromate may be seen from the slopes of the graphs in Fig. 3 (left). The highest response or slope is given by flour C, a baker's special or a patent type. The lowest response is given by the baker's strong flour D which was a clear type and was of lowest quality. It may be of interest to add that the flours giving the highest response had (consequently) the lowest optimum bromate requirement in the baking test (Table I).

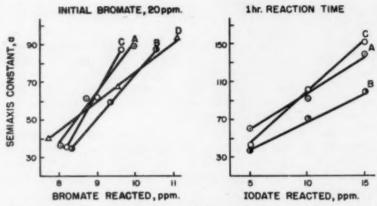


Fig. 3. The improver response of several flours as shown by plots of the semiaxis constant against bromate reacted in doughs containing initially 20 p.p.m. bromate (left); and the semiaxis constant against iodate reacted in doughs after a reaction time of 1 hour (right).

while the flour giving the lowest response had the highest bromate requirement.

Two main factors appear to determine the bromate response as defined here. Of these the semiaxis constant a does not show a large variation for the flours tested here. The rate of bromate loss in dough appears, therefore, to be the major factor. The bromate response (a/c) includes both of these.

Variation of Semiaxis Constant a with Iodate Concentration c. The use of iodate rather than bromate as a reagent for assessing the improver response of flour or dough is somewhat simpler. The reason for this is that the amount of bromate reacted in dough depends upon the reaction time and must be determined chemically. For iodate, because of its fast reaction, the amount reacted can be simply taken as the amount of iodate initially added to dough.

Data on the response of flours A, B, and C to iodate are shown in the right half of Fig. 3. (No more flour D was available at this time.) The initial amounts of iodate added (assumed to be the same as the amount of iodate reacted) were 0, 5, 10, and 15 p.p.m. A uniform reaction time of 1 hour was given for all doughs. It will be seen that the response to iodate was the highest for flour C, followed by A and B. This order is the same as that obtained in experiments with bromate and shown in the left half of Fig. 3.

Discussion

The primary aim of the present investigation was to look into the

feasibility of describing the improver response of flour or dough from a more fundamental point of view than has been possible thus far. To this end, additional support has been presented for the validity of the assumption that the semiaxis constant is a basic rheological parameter. This support is based on the analogous linearity between the semiaxis constant against reaction time, on the one hand, and the bromate reacted against reaction time, on the other. The improver response was then examined as the change in the semiaxis constant per unit amount of improver reacted in the dough. While bromate and iodate may react somewhat differently in dough, either may be used in evaluating the improver response. On the basis of data obtained for four different flours, the rate of bromate reaction in dough and the protein content of flour appear to be strongly related to the improver response. Moreover, those flours that showed a high bromate response had also a low optimum improver requirement in the baking test, and conversely.

One is, of course, tempted to speculate on the practical implications of the results obtained in this study, and there are interesting implications. However, the experimental work was done with simplified flour-water-salt doughs mixed in nitrogen and on only a few flours. The emphasis has been on the basic aspects. This study is, therefore, more appropriately regarded as a prerequisite fundamental phase for a more practically oriented future study of improver response.

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SOME AROMATIC COMPOUNDS PRESENT IN OVEN GASES1

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ABSTRACT

The only compounds identified in condensates from bread oven vapors were ethanol, acetaldehyde, and acetic acid. Quantitative analysis of a representative condensate from twelve 1-lb. loaves yielded these values: ethanol, 3.9 g.; acetaldehyde, 55 mg.; acetic acid, 58 mg. A synthetic mixture of this composition, and several variants of it, failed to approximate the aroma of the oven gases. Mixtures to which furfural and acetylmethylcarbinol were added approached the natural aroma much more closely; an "optimum" composition is given. It is manifest that presently unidentified compounds, probably in trace amounts, must determine in large measure the aroma of oven vapors.

In a previous paper (6), some of the readily volatile compounds thought to be implicated in the aroma of fresh bread were characterized. Attempts to simulate the bread aroma with mixtures of these substances were not notably successful. It was thought distinctly possible that unidentified trace constituents might be of major importance in determining the aroma of bread, and further that the extremely appealing aroma of oven vapors might denote the presence of these unidentified substances in greater proportions. Baker and associates (1,2) reported the presence of a remarkable variety of organic compounds in bread oven vapors. In the present study, an attempt was made to collect as much as feasible of such substances by a variety of means, including condensation in cold traps and absorbing individual classes of compounds in suitable reagent solutions.

Materials and Methods

The first requisite was a source of oven gases, which would preferably be uncontaminated by gaseous products of combustion and be available on a large scale. In the bread shop of the American Institute School of Baking the indirect-fired Petersen "70" oven was fitted with a high-capacity condenser as follows:

An aluminum sampling tube, $1\frac{1}{2}$ in. o.d., was inserted through the wall of the 14-in. duct which vents the baking chamber of the oven, as close as possible to the oven itself. The sampling tube connected with a condenser comprising 9 ft. of $1\frac{1}{2}$ -in. o.d. aluminum tubing jacketed with 8 ft. of $2\frac{1}{2}$ -in. galvanized steel pipe. The ends of the

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pipe were threaded to take pipe caps, and the latter were drilled out to pass the aluminum tubing. Neoprene stoppers were bored with 1½-inch holes and slipped over the ends of the aluminum tube, with their narrow ends just entering the reamed ends of the pipe. When the drilled pipe caps were drawn up snugly, the stoppers were seated firmly and sealed the jacket perfectly. The jacket was drilled near the ends and ordinary pipe nipples were brazed on to receive garden-hose couplings for circulation of cooling water. A curved and a straight section of 1½-in. tubing, joined with short sleeves of Tygon tubing, led the condensate into a wide-mouthed 5-gal. bottle through a thick Neoprene gasket, into which was also inserted a ½-in. copper tube serving as a vacuum line. The latter was fitted with a ½-in. brass gate valve to control the amount of vacuum applied and prevent excessive entrainment of spray. An industrial vacuum cleaner was found to provide ample suction.

The 5-gal. receiver bottle was at first packed in chopped ice during operation; but this was found to accomplish little in trapping highly volatile materials. It had been planned to insert a Dry Ice trap between the receiver and the vacuum source, until calculation showed that at the normal range of vapor flow rates the dimensions of such a trap, to be of any benefit, would have to be impossibly large. Adding about 10 lb. of chopped ice inside the bottle before beginning a run helped to chill the condensate for a short time, at the cost of diluting the condensate considerably. In normal operation, the exhaust of the vacuum cleaner discharged a considerable concentration of acetaldehyde vapor; this seemed to be the only compound being lost to any great extent.

For more precise quantitative work, baking was done in a Blue M Model OV 18S electric laboratory oven. One of the two top vents was plugged; the second was fitted with a vapor draw-off tube leading to the condensers or scrubbing solutions. Mild vacuum was applied in either case to assure that the oven gases would be drawn into the collecting devices; normal air leakage around the door seal assured that the pressure inside the oven would not be measurably below atmospheric. Condensation of the vapors was done in a system comprising a water-cooled Friedrichs condenser, two trap flasks surrounded with ice and salt, and a large Dry Ice trap. On one run a liquid nitrogen trap was added to the foregoing, but did not condense anything that might have passed through the Dry Ice stage.

No attempts were made to absorb organic acids from the vapors by means of alkaline solutions, with one exception noted below, since the acids are all readily condensable. Carbonyl compounds in the vapors from the electric oven were absorbed by bubbling the vapors through a long tube filled to a depth of around 18 to 24 in., either with 2,4-dinitrophenylhydrazine in 2N hydrochloric acid or with dilute sodium bisulfite solution. On one occasion, barium hydroxide solution was used to trap the volatile acidic material for identification. Using the large gas-fired oven, no attempts were made to scrub the vapors.

Qualitative analysis of the oven-vapor constituents generally followed the procedures described in a previous paper (6). After separation of carbonyl compounds as their 2,4-dinitrophenylhydrazones and acids as the sodium or barium salts, the remaining material from the condensate was saturated with sodium sulfate and extracted at 0°C. with ethyl chloride. The ethyl chloride (b.p. 12°C.) was distilled off through a short Vigreaux column without the use of heat, and vapor-phase chromatography was used to study the residual organic material. Samples were applied to a 10-ft. column containing a polyester (diethylene glycol succinate) on Chromosorb as the packing. The instrument (Aerograph Model C-100) was operated at 100°C, with a helium carrier gas flow rate of 25 ml. per minute.

Quantitative anlayses were simplified considerably when it was determined that ethanol, acetic acid, and acetaldehyde were by far the most predominant components of the oven vapors, apart from water. Ethanol was estimated by oxidation with acid dichromate and measurement of excess dichromate, using the micro-method of Neish (4). Acetic acid was estimated by ordinary titration with 0.05N potassium hydroxide solution, using a mixed phenolphthalein-thymolphthalein indicator. Acetaldehyde was estimated by its bisulfite-binding capacity, using both the unmodified method of Neish (4) and a variant in which the excess unbound bisulfite rather than that bound by the aldehyde is determined. The details in which this latter method differs are pointed out under "Results."

Results

Examination of Condensates. During a typical run with the large oven, about 2 gal. of condensate were collected in one morning. Unfortunately, the oven was opened at intervals to load and unload, so that there was considerable loss of vapors through the doors; quantitative collection was obviously out of the question. When a day's run was completed, the condensate was transferred to glass-stoppered flasks which were filled completely and stored in a refrigerator. Five gallons of condensate were pooled, and distillation at atmospheric pressure yielded about 11/4 gal. of distillate plus a completely odor-

less residue. The distillate was redistilled through a Vigreaux column fitted with a total-reflux, partial-takeoff stillhead, yielding 1,400 ml. of distillate and an odorless residue. This second distillate was then distilled again through the same apparatus to yield 300 ml. of highly odorous distillate and an odorless residue. This final distillate was saturated with sodium sulfate, chilled to 0°C., and extracted with ethyl chloride.

Individual portions of the extract were treated with 2,4-dinitrophenylhydrazine in 2N hydrochloric acid; 3,5-dinitrobenzoyl chloride in toluene; and p-phenylphenacyl bromide, according to standard procedures for preparation and identification of organic derivatives of carbonyl compounds, alcohols, and acids respectively (3,5). The results indicated that only acetaldehyde, ethanol, and acetic acid were present.

Using the electric oven, it was felt that the findings would be more trustworthy. For example, one run using the large oven yielded an apparently wide range of volatile compounds, which proved to be due to prior lubrication of the oven. Runs were made using the electric oven with the system of condensers described above, and also using the scrubbing tower filled at different times with solutions of 2.4-dinitrophenylhydrazine, sodium bisulfite, and barium hydroxide. In the case of the condensates the procedure described under "Methods" was followed, and the sole 2,4-dinitrophenylhydrazone detected was that of acetaldehyde, while the sole acid isolated as its barjum salt proved to be acetic acid, by its unique p-phenylphenacyl ester and by its single peak on the vapor-phase chromatograph. The ethyl chloride extract vielded a single peak on the polyester column, corresponding to ethanol. Confirmation was obtained by preparing the 3,5-dinitrobenzoate and checking against an authentic sample. The scrubbing tower was used purely in the hope of trapping organic volatiles other than those noted, but without success.

Quantitative Analysis of Condensate. (1) Acetic Acid. The total condensate collected (using Dry Ice and liquid nitrogen traps) from the baking of 12 loaves in the electric oven was made to 250 ml. and 25-ml. aliquots were titrated with potassium hydroxide solution.

KOH used: 2.04, 2.03 ml. at 0.0472NAcetic acid in aliquots: $2.04 \times 0.0472 \times 60 = 5.8$ mg. Total acetic acid in condensate: $5.8 \times 10 = 58$ mg.

(2) Ethanol. A 10-ml. portion of the condensate was diluted to 100 ml., and 2.0-ml. aliquots were treated with 10.0 ml. of the dichromate reagent (0.0400N potassium dichromate in 18N sulfuric acid). The titers on duplicate aliquots were 0.270 and 0.269 meq.

of dichromate, giving ethanol contents of 3.1 mg. in the aliquots, 250×10

or
$$3.1 \times \frac{250 \times 10}{2 \times 1000} = 3.9$$
 g. total.

(3) A retaldehyde. Using the unmodified Neish method (4), and titrating bisulfite regenerated from the complex with 0.05N iodine, meq. acetaldehyde per 10 ml. aliquot = 0.0357. In total condensate,

acetaldehyde =
$$\frac{250}{10} \times 44 \times 0.0357 = 39.3 \text{ mg}.$$

This method was suspected of yielding low results, as the release of bisulfite from the aldehyde complex by sodium bicarbonate seemed very gradual. Using 2 ml. of 1% sodium bisulfite solution (about 0.1M) and adding 10.0 ml. of 0.05N iodine to destroy the excess, the unconsumed iodine was back-titrated with 0.04N thiosulfate. Milliequivalents acetaldehyde per 10 ml. aliquot = 0.0501, which

gives a total content of
$$\frac{250}{10} \times 44 \times 0.0501 = 55$$
 mg.

It must be noted that these values are representative only of the particular condensate used; repetitions of these analyses on other condensates from the electric oven indicated only order-of-magnitude agreement with the ones given here. Doubt is thereby thrown on the extent to which the sampling of oven gases is quantitative. However, these order-of-magnitude results were considered valid enough to permit experiments on simulating the aroma of oven gases.

"Synthetic Oven Vapors." Using the above proportions as a datum, several mixtures of 95% ethanol, acetaldehyde, and glacial acetic acid were made up. Holding the amount of ethanol constant, each of the other two components was varied, using the proportion cited and also one-half and twice this proportion. Aliquots of the nine mixtures were pipetted into varying volumes of hot water, and the odors were noted. In no instance did the odor resemble the oven vapors at all closely; the three organic compounds were clearly identifiable.

As a matter of curiosity, the mixture containing half the above proportion of acetaldehyde and the normal proportion of acetic acid was modified by additions of various amounts of acetylmethylcarbinol and furfural. Diacetyl was tried and rejected when it was found that its odor was too distinctive, even in the minutest traces. While no combination of acetylmethylcarbinol and furfural yielded an aroma very close to that of the oven gases, the improvement over the simple ethanol-acetaldehyde-acetic acid composition was notable. The optimum concentrations, arrived at by trial and error, were:

Ethanol	4.0 g.
Acetaldehyde	10.0 mg.
Acetic acid	40.0 mg.
Furfural	20.0 mg.
Acetylmethylcarbinol	6.0 mg.

One milliliter of this mixture in about 150 ml. of warm water produced a very pleasant aroma, though not quite that of the oven gases.

Discussion

In the experiments described, the apparent composition of the oven gases from the baking of white bread was very simple. That this composition does not account fully for the aroma is manifest from the attempts to reproduce the aroma synthetically, especially in view of the improvement afforded when furfural and acetylmethylcarbinol were incorporated. It must be concluded that the analytical methods used here did not detect traces of extremely important substances which, together with those which were detected, produce the characteristic and appealing aroma of baking bread. In Baker's studies (1,2), a large variety of substances was reported as occurring in the oven vapors; the work reported here failed to detect most of these substances, even with the use of a commercial-type oven to provide large volumes of condensed vapors.

Since the bulk of the condensable material in the oven vapors is water, efficient concentration of the organic components requires either very thorough extraction of the aqueous condensate or selective removal of water from the vapors. Experiments on the preferential sorption of water vapor on a glycerol-impregnated porous granular support are under way, using dilute aqueous solutions of the lower aliphatic alcohols, aldehydes, and acids as "synthetic" vapor sources.

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THE VOLATILE ORGANIC ACIDS FOUND IN DOUGH, OVEN GASES, AND BREAD¹

LAZARE WISEBLATT

ABSTRACT

The volatile aliphatic acids found in fermented dough, bread, and oven vapors, with their concentrations as estimated by vapor-phase chromatography, are (milligrams per thousand grams of flour basis): acetic, 198, 150, 15; n-butyric, 23.1, 10.6, 0; isovaleric, 13.1, 6.1, 0; n-caproic, 6.2, 0, 0. The acids comprise only those which are steam-distillable from fermented dough and bread extracts, and that found in oven vapors. The amounts in the bread and oven gases do not add up to the corresponding amounts in dough, leading to speculation regarding the fates of the dough acids during baking.

The nature of the contribution of volatile aliphatic acids to bread aroma is not well understood, and in fact the identities, amounts, and sources of these acids have received inadequate study heretofore. The present study was undertaken as part of a broad investigation into various aspects of bread flavor, some results of which have been presented elsewhere (2,3).

Materials and Methods

The doughs used in this work were made up from this formulation:

Flour	(N	ľ	V	V	Ī	2	ıt	c	n	ıŧ)						į				1000 g.
Yeast												,										30 g.
Sucrose	e																				0	60 g.
																						20 g.
																						20 g.
Water																						630 ml.

The same formulation was used whether the fermented dough or the bread was to be analyzed. After mixing in a Hobart A-120 mixer fitted with a McDuffee bowl and fork, fermentation was carried out at 30°C. (86°F.). Two punches were given, at 75 and 105 minutes, and the doughs were taken at 135 minutes (from the start of the mixing). If the dough was to be studied as such, it was scaled, molded, and proofed normally in pans, and then the proofed dough was treated as described below. Otherwise, the doughs were baked in a Blue M Model VO 18S electric laboratory oven. This oven has a low heat-recovery rate, so it was loaded at 255°C. (491°F.); the

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oven temperature quickly fell to about 203°C. (397°F.) and recovered slowly to about 230°C. (446°F.) in 30 minutes. The loaves baked out satisfactorily in the 30 minutes. One set of loaves provided material both for the oven-gas analysis and analysis of the bread itself, when the gases were drawn into an efficient Friedrichs condenser followed by an ice-salt cold trap.

Recovery of Acids from Dough. The fermented and proofed dough from 1,000 g. of flour was stirred with 2 liters of reagent-grade acetone until completely disintegrated, and filtered with mild vacuum on a large Büchner funnel. The cake was ground up with 1 liter of acetone and filtered again; this sequence was repeated three times. The combined filtrates were distilled through an 18-in. Vigreaux column to remove most of the acetone, leaving an aqueous residue with a layer of liquid fat on top. The fat layer was free of volatile acids.

The residue was diluted with water, the fat layer drawn off as completely as possible, and the aqueous phase was distilled rapidly, with addition of water to maintain the volume in the still at over 1 liter. Two liters of distillate were collected, and were titrated with saturated barium hydroxide solution to the phenol red end-point (pH 8.4). The solution containing the barium salts of the acids was vacuum-evaporated to dryness, using a rotary evaporator. The dry residue of barium salts was taken up in sufficient 2M sulfuric acid (calculated from the titer) to ensure a final pH below 2.5, and the mixture, after saturation with solid sodium sulfate, was extracted repeatedly with reagent-grade diethyl ether to remove the free, un-ionized acids. The ether extract was evaporated carefully under an air condenser, and was then diluted up to 5.00 ml. with ether for analysis.

Recovery of Acids from Bread. The same scheme as detailed above was followed, the fresh bread having been previously disintegrated by the blades of a Waring Blendor. (CAUTION: not more than about 2 oz. at a time!) The quantities given are suitable for three 1-lb. loaves of bread. Even with the previous disintegration, the filter cake from bread tended to be loose, and the use of a rubber dam over the filter funnel is recommended to improve the expression of occluded acetone. The suction flask was also chilled in an ice bath to improve the vacuum, and hence the pressure applied to the filter cake.

Recovery of Acids from Oven Gases. The oven-gas condensate was made alkaline as above with barium hydroxide solution, and subsequent steps followed the scheme described.

Calibration of Gas Chromatograph. The instrument used was the Aerograph Model A-100, with a 4-filament thermal conductivity de-

tector cell. Filament power (200 ma.) was supplied by a Beckman model 2695 hydrogen-lamp power source, whose output was dropped through a heavy voltage-divider resistor and restabilized at about 13 volts by a 10-watt zener diode (Texas Instruments Co., Type 1N 1816) shunted across the input to the filament circuit. Used in this way, the zener diode dissipated about 1.3 watts to the instrument case, on which it was mounted by its anode stud using mica insulating washers. The column used throughout was a stainless steel column, 10 ft. by 1/4 in., packed with a silicone-stearic acid mixture on an inert support, as described by James and Martin (1). It was operated at 120°C., rather than the 137°C. used by these workers, and showed much improved stability at the lower temperature. Good resolution of the peaks was obtained for the normal alphatic acids in the C2 to C6 range, although tailing prevented measurement of total peak areas. It was also possible to distinguish between the retention times of the normal and iso C4 and C5 acids, although in mixtures of the normal and iso acids single, broadened peaks were obtained.

To calibrate the column, 10% (w/v) solutions of acetic, propionic, isobutyric, and isovaleric acids in benzene were prepared. Using the standard conditions of helium flow rate, 120°C. and 25 ml. per minute, samples of each solution were injected from a 50 μl. Ham-

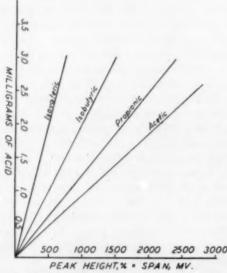


Fig. 1. Calibration plots for estimating volatile acids by vapor-phase chromatography.

ilton syringe. Because of tailing, the peak heights only were recorded. Figure 1 shows the plots of mg. acid against (peak height × span) for the various acids, using samples of 5, 10, 15, etc. microliters of the solutions. The "span" referred to is the number of millivolts required to drive the recorder pen to 100% of scale at the particular sensitivity attenuation used. Thus all the samples are compared on a common basis, independent of the individual sensitivity settings used.

Although complete runs were not done with n-butyric, n-valeric, and n-caproic acids, it was noted that the calibration line for n-butyric would fall very nearly midway between those for propionic and isobutryric, while that for n-valeric almost coincided with isobutyric and that for n-caproic with isovaleric. Interpolation, if necessary, would be sufficiently accurate for the reproducibility of this method of analysis.

Several mixtures of known C₂ to C₆ acids, made up accurately by weight, were applied to the gas chromatograph, and the amounts of the component acids estimated from the calibration plots. The accuracy for acetic and propionic acids was excellent, normally better than 5%, and decreased as expected for the higher acids, whose peaks are lower and broader. The results cited below show, by the agreement between gas chromatographic and titration data, that the procedure used is of practical utility, although the theoretical basis for using peak heights alone is weak. Identities of the acids were based on retention times.

Analysis of Acids from Dough, Oven Gases, and Bread. The ether solutions of the volatile organic acids were applied to the gas chromatograph column, determining by trial the size of sample and the sensitivity required to record the detectable peaks adequately. It was usually necessary to reduce the sensitivity by a considerable factor to keep the acetic acid peak on scale, and to increase it again to get adequate peak heights for the other acids. The peak heights and spans were noted, and the equivalent milligrams of each were read or interpolated from Fig. 1.

Results

Volatile Acids in Dough. By titration with barium hydroxide, the acidity of the distillate from a fermented and proofed dough (1,000 g. of flour) was 3.73 meq. From the peak heights of the acids, the sample volumes, and their retention times, the following data for the total acids were obtained:

	mg.		meq.
Acetic	198.0	or	3.3
n-Butyric	23.1	or	0.26
i-Valeric	13.1	or	0.13
n-Caproic	6.2	or	0.05
	Т	otal	3.74

The agreement with the titration value is excellent.

Volatile Acids in Oven Gases. As reported elsewhere (2), only acetic acid was found. On the basis of 1,000 g. of flour, the amount of acetic acid was 15 mg.

Volatile Acids in Bread. The acidity of the distillate from bread representing 1,000 g. of flour, by barium hydroxide titration, was 2.79 meq. By gas chromatography, the following data were obtained:

	mg.		meq.
Acetic	150.0	or	2.5
Butyric	10.6	OT	0.12
i-Valeric	6.1	or	0.06
	Т	otal	2.68

The agreement was not as good as found for dough. Further, no caproit acid was detected in the bread.

Discussion

If we add the acetic acid found in bread to that found in the oven gases from the same batch, we obtain a total of 150+15=165 mg. The equivalent dough contained 198 mg. Comparing the n-butyric and isovaleric acid contents for the dough and bread, we see a reduction of rather more than half of each acid. It would be pure speculation to assume that the differences represent esterification of these acids during baking; the conditions are probably more conducive to hydrolysis. The general procedure used in these studies was evolved after trial and rejection of other extraction methods, including water extraction of the dough and bread at pH 2 or lower. Separation and estimation of the acids by partition column chromatography showed no notable advantages in selectivity or accuracy over the vapor-phase chromatographic method, while being far slower and more costly in material and solvents.

What these analyses may imply as regards the flavor of bread will depend to some extent on how the amounts of the various acids change during the storage of bread, and how these changes correlate with flavor alterations. The volatile acids composition of a fermented dough can be shown to vary greatly, depending not only on the physical conditions but also on the ingredients. Not the least

important of these ingredients is the yeast; unpublished preliminary experiments in this laboratory, using simple liquid brews fermented by different commercial baker's yeasts, have shown striking differences in the volatile acids compositions, and also in the aromas of the brews.

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SULFHYDRYL LOSSES DURING MIXING OF DOUGHS: COMPARISON OF FLOURS HAVING VARIOUS MIXING CHARACTERISTICS¹

H. A. SOKOL, D. K. MECHAM, AND J. W. PENCE

ABSTRACT

Twelve flours of varied types were made into doughs in a farinograph and their sulfhydryl contents determined after 2, 5, 10, and 20 minutes of mixing. Sulfhydryl losses varied after 20 minutes of mixing from 38 to 64% of the original sulfhydryl content. An initial short period (2 to 5 minutes) of rapid decrease in sulfhydryl content was observed with most of the flours. With continued mixing, relatively large differences were found in the rate of decrease; treated by first-order kinetics, values for the rate constant for the 5-to-20-minute period ranged from 6 to 36 minutes-1.

Doughs from three flours (including two durums) were mixed with added flour albumin. The initial decrease in sulfhydryl content then was more rapid, while the rate of loss from 5 to 20 minutes was decreased.

Stability values taken from farinograms correlated well with the sulfhydryl-loss rate constant for three hard red spring, three white, and two hard red winter wheat flours, and for the two durum flours supplemented with albumin. The nonsupplemented durums and two hard red winter wheat flours giving atypical farinograms did not show this relationship.

With two flours, the rate of loss of sulfhydryl groups in 10:1 water-flour suspensions decreased much more slowly than in the corresponding doughs.

The sulfhydryl content of straight-grade flour is about 1 μ eq. per g. of flour (2,3,9). Despite this relatively low concentration, reagents

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that react specifically with sulfhydryl groups bring about marked changes in the physical properties of doughs during mixing (5). Such observations indicate that sulfhydryl groups may be an important factor in the wide natural variations found in the mixing characteristics of flours.

A considerable part of the sulfhydryl groups in some flours is lost during mixing of a dough in air and oxygen, presumably by oxidation (2,8). The available reported information, however, is limited to a few flours. If sulfhydryl groups do contribute to the mixing characteristics of different flours, it was thought that the rates of decrease of sulfhydryl content during dough mixing might be found to differ. The work reported here was undertaken to investigate this possibility.

Materials and Methods

The flours were unbleached, straight-grade, and experimentally milled, except for the durum and commercial hard red spring flours which were commercially milled. Protein and ash contents and the sources of the flours are given in Table I.

TABLE I
ASH AND PROTEIN CONTENTS OF FLOURS (DRY BASIS)

FLOUR	Source	Ass	PROTEIN
		%	%
Durum No. 1	California	0.74	11.0
Durum No. 2	Dakotas	0.93	12.8
Mida, HRS	North Dakota	0.41	14.7
Lee, HRS	North Dakota	0.39	17.5
Commercial, HRS	Montana	0.45	15.6
Baart, SWS	Washington	0.62	13.0
Idaed, SWS	Washington	0.53	14.2
Elgin, Club	Washington	0.58	13.8
UN No. 1, HRW	Nebraska	0.49	12.8
UN No. 2, HRW	Nebraska	0.54	14.4
Chevenne, HRW	Montana	0.45	16.5
Wasatch, HRW	Montana	0.44	13.6

Sulfhydryl determinations usually were carried out on lyophilized doughs. Flour and water were mixed for 2, 5, 10, and 20 minutes in a 50-g. farinograph bowl at 29°C. Immediately after mixing, each dough was compressed between blocks of dry ice, the frozen dough was lyophilized, and the dried dough ground (Wiley mill) through an 80-mesh screen. Previous work has shown that grinding itself does not cause a loss of sulfhydryl. After exposure to the atmosphere for one day, portions of the dried dough were dispersed in a buffer-urea

medium and were titrated amperometrically with silver nitrate (9). Each individual sulfhydryl value reported below represents at least three titrations of differing sample sizes.

Results with suspensions (Elgin and Lee flours) were obtained as follows: Two grams of flour were added to 20 ml. of distilled water at room temperature (about 25°C.) during vigorous mixing with a magnetic mixer. This mixing lasted for about 30 seconds in every case. Then the mixer was set at a constant moderate speed and the mixing was timed for 5, 10, or 20 minutes at room temperature. A 2-g. sample was used for each of the three mixing times. Precautions were also taken to prevent heating of the suspension by the mixer. After the suspension was mixed for the proper length of time a 6-ml. aliquot was withdrawn and added immediately to a buffer-urea-salt solution; titration was then performed as usual (9).

The albumin sample was prepared from the commercial hard red spring wheat flour as directed by Pence and Elder (6); the yield was 0.37% of the flour. Albumin-supplemented doughs contained 0.37% (based on flour weight) of albumin added to the flour in the farinograph bowl before mixing. The sulfhydryl content of the albumin was $5.7~\mu eq.$ per g.; its contribution to the titer of a dough was neg-

ligible.

Previous work (10) has shown that a loss of about 5 to 10% of residual sulfhydryl groups occurred during the lyophilization process. The values presented in this paper therefore will be slightly lower than would be found with undried doughs. The error is consistent and in one direction, however, and should not affect the trends observed.

Farinograms were obtained on flour-water doughs, and the conventional technique was used (50-g. bowl).

Results and Discussion

Sulfhydryl losses during mixing are given in Table II for ten flours of widely varied type and source. Considerable differences in the rates of loss were found; this is more readily apparent in Fig. 1, in which data are plotted for four flours representative of different types.

In all of the doughs mixed in air the sulfhydryl content decreased most rapidly during the initial stages of mixing, although the actual losses in the first 2 minutes ranged from 0.10 to 0.35 μ eq. of sulfhydryl per g. As mixing continued, a marked change in the rate of loss appeared in most cases beyond 2 minutes of mixing. Furthermore, it may be noted that the Pacific Northwest wheat flours (Idaed, Baart, Elgin) known typically to have poor mixing tolerance all showed low

TABLE II

SULFHYDRYL CONTENTS OF FLOURS AND DOUGH AFTER
2, 5, 10, AND 20 MINUTES OF MIXING IN AIR

FLOUR	-SH CONTENT MINUTES OF MIXING											
	Qu.	2	5	10	20							
	µeq/g	μeq/g	meq/g	meg/g	μeq/g							
Baart	0.96	0.61	0.60	0.56	0.54							
Elgin	0.76	0.63	0.53	0.47	0.45							
Idaed	0.82	0.63	0.60	0.55	0.48							
Commercial	1.12	0.92	0.68	0.64	0.52							
Mida	0.80	0.57	0.49	0.51	0.35							
UN No. 2	0.82	0.71	0.67	0.59	0.51							
UN No. 1	0.69	0.59	0.55	0.48	0.37							
Lee	0.97		0.64	0.58	0.42							
Wasatch	0.75	0.60	0.62	0.50	0.41							
Chevenne	0.98	0.69	0.62	0.60	0.37							

a Whole flour.

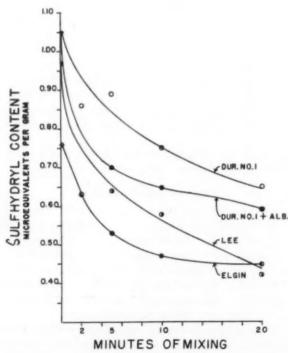


Fig. 1. Sulfhydryl contents of doughs after various mixing times.

rates of sulfhydryl loss beyond 5 minutes mixing.

Albumin Supplementation of Durum Doughs. Sulfhydryl contents of doughs mixed from two durum flours also were determined. The decreases in sulfhydryl content showed a less pronounced change of rate in the 2-to-5-minute range than occurred with other flours (Fig. 1). Pence et al. (7) have shown that durum flours characteristically contain less of the beta-albumin protein components than common or club wheats. An albumin preparation from a common wheat flour therefore was added to the durum flours (and to the Montana Cheyenne) to determine whether the rate of loss of sulfhydryl groups would be affected. The results are given in Table III, and those for Durum No. 1 are shown in Fig. 1. With all three flours, the albumin addition increased the rate of loss of sulfhydryl groups in the early stages of mixing; the change in the curve was then more abrupt and the decrease during the remaining mixing was slower than in the control.

TABLE III

SULFHYDRYL CONTENTS OF DURUM FLOURS, MONTANA CHEYENNE FLOUR, AND THEIR
DOUGHS BEFORE AND FOLLOWING ALBUMIN SUPPLEMENTATION AFTER
2, 5, 10, AND 20 MINUTES OF MIXING IN AIR

FLOUR	-SH CONTENT MINUTES OF MIXING				
	04	2	5	10	20
	Heq/K	µeq/g	µeq/g	meg/g	μeq/g
Durum No. 1	1.05	0.86	0.89	0.75	0.65
Durum No. 1 + alb.	1.05		0.70	0.65	0.59
Durum No. 2	0.95	0.84	0.72	0.62	0.46
Durum No. 2 + alb.	0.95		0.65	0.58	0.55
Chevenne	0.98	0.69	0.62	0.60	0.37
Chevenne + alb.	0.98		0.51	0.39	0.35

a Whole flour.

Reaction Rate Constants. In order to provide a numerical comparison of the rates of sulfhydryl loss for possible correlation with mixing curve characteristics of the flours, reaction rate constants were determined for the 5-to-20-minute period of mixing. First-order kinetics were used. This approach appeared preferable to the calculation of percent loss of sulfhydryl groups after a specified time of mixing, because the latter value would be affected by the original sulfhydryl contents which varied by a factor of 1.6 (0.69 to 1.12 μ eq. per g. flour).

Treatment of the rates of loss of sulfhydryl groups as first-order undoubtedly represents an oversimplification. Even in a logarithmic plot, the original sulfhydryl contents of the flours usually do not fall into line, and the 2-minute values do not in several cases. On the other hand, when only the 5, 10, and 20-minute values are plotted, a zero-order plot (rectangular coordinates) is nearly as satisfactory as a first-order plot. For purposes of comparison and correlation, however, first-order treatment seemed more nearly suitable, since a wider range of first-order rate constant values were obtained.

Examples of the first-order plots, chosen to show cases of relatively large and small deviations of experimental points from the calculated line, are shown in Fig. 2. Reaction rate constants for all the flours are given in Table IV. The k values are distributed throughout the range from 6.5 to 36.4×10^{-3} minutes $^{-1}$.

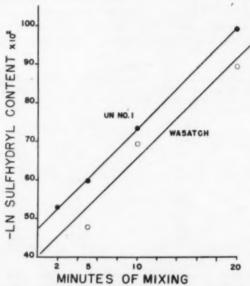


Fig. 2. Time of mixing vs. natural logarithm of the sulfhydryl content for UN No. 1 and Wasatch flours.

Correlation with Mixing Curve Characteristics. By examination of the rate constants, it was apparent that in general the soft wheat flours gave low values of k, while the hard red spring wheat flours gave markedly higher values. Farinograph curves for these flours showed in most cases the differences in mixing characteristics typical of the various classes of flour. When peak time, drop-off and stability were measured (1), the reaction rate constants seemed related most consistently to the stability values. The stability values are given in Table IV, together with the rate constants.

In general, as rate constants increase, stability values increase. The two durum and the two Montana hard red winter wheat flours are exceptions; these flours gave high rate constants but had poor stability

TABLE IV
DOUGH STABILITIES AND SULFHYDRYL-LOSS RATE CONSTANTS FOR
VARIOUS FLOURS AFTER 20 MINUTES OF MIXING IN AIR

FLOUR	STABILITY TIME	RATE CONSTANT	
	minutes	minutes-1 ×100	
Durum No. 1	0.9	20.0	
Durum No. 1 + alb.	1.1	11.2	
Elgin	1.2	10.0	
Baart	1.4	6.5	
Wasatch	2.2	26.5	
Cheyenne	2.5	36.4	
Cheyenne + alb.	2.5	23.0	
Durum No. 2 + alb.	4.0	10.3	
Durum No. 2	4.9	29.8	
Idaed	4.9	14.7	
Commercial	8.0	18.3	
Mida	9.7	24.6	
UN No. 2	12.8	17.7	
UN No. 1	13.9	25.3	
Lee	13.3	28.7	

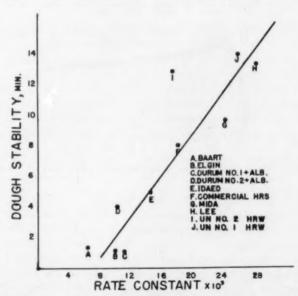


Fig. 3. Correlation of dough stabilities with apparent first-order rate constants.

to mixing in the farinograph. Supplementations of the durum doughs with the hard red spring albumin had little effect on their stability but brought the rate constants into line with the general trend. In fact, the correlation coefficient for rate constant vs. mixing stability is +0.92 ** if the supplemented durum values are used and the two Montana flours are excluded. This relationship is illustrated in Fig. 3. The very low stability value of the Cheyenne flour is not typical for this variety of wheat. Farinograph curves to illustrate the nature of the Cheyenne and Wasatch flours are shown in Fig. 4. No reason for their failure to fit into the rate constant vs. mixing stability relationship can be given, however.

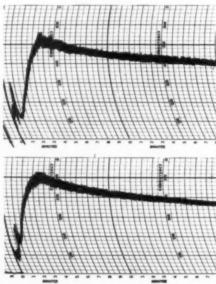


Fig. 4. Top curve, farinogram of Cheyenne hard red winter flour, 72.2% absorption at 14% moisture. Bottom curve, farinogram of Wasatch hard red winter flour, 70.4% absorption at 14% moisture.

Flour Suspensions. In previous work (10), the reactivity of the sulfhydryl groups of flour as determined in suspensions in urea solution covered a considerable range; some groups reacted fairly rapidly while others appeared to be inaccessible to sulfhydryl-blocking reagents. Iodoacetamide, for example, blocked fewer sulfhydryl groups than were lost during mixing of doughs in air in the present study. This suggested that the action of dough mixing renders sulfhydryl groups more accessible than does the stirring of a flour suspension

without dough development. Possibly a related suggestion is that made by Bushuk and Hlynka (4), i. e., that the reaction of bromate in doughs is controlled by the physical process of mixing a dough.

Therefore the rate of loss of sulfhydryl groups in a flour-water (1:10, w/v) suspension was determined with the Elgin and Lee flours. Losses were much slower in suspensions than in doughs; the data are given in Table V. In view of the very low rate of loss beyond 10 minutes' mixing, it appears that sulfhydryl groups fail to become available for reaction rather than that the decreased rate results from lack of oxygen or from dilution of some other flour constituent reacting with sulfhydryl groups.

TABLE V
SULFHYDRYL CONTENTS AFTER 5, 10, AND 20 MINUTES OF MIXING AND RATE
CONSTANTS OF SULFHYDRYL LOSS FOR FLOUR-WATER SUSPENSIONS

	3	-SH CONTENT SINUTES OF MIX		RATE CONSTANT
	5	10	29	
	µeq/g	meq/g	meg/g	minutes-1 ×10 ⁸
Elgin Lee	0.66 0.84	$0.62 \\ 0.77$	0.62 0.72	3.5 9.8

General Discussion

The suggestion that sulfhydryl groups participate in some way in determining the mixing characteristics of flours was based on the effects of sulfhydryl-blocking reagents on dough properties (3). The observations presented in this paper support such a suggestion with a different kind of evidence. Doughs prepared from flours of varied types were found to differ with respect to the rate of loss of sulfhydryl groups during the course of mixing, demonstrating a natural variation among flours. The rate can be altered by addition of albumin components, suggesting one possible basis for the natural variation. Finally, a tendency for mixing stability and rate of loss of sulfhydryl content to vary together was observed with a limited number of flours, although the exceptions emphasize the need for more investigation. Nevertheless, some relationship between sulfhydryl groups and dough development and stability to mixing seems fairly certain.

It was suggested earlier (5) that the development and stable portions of dough mixing curves may represent the periods when sulfhydryl groups are present in quantities large enough to promote appreciable sulfhydryl-disulfide interchange; with adequate interchange the building-up of strains in the dough is reduced and its breakdown deferred.

The results in the present paper indicate that sulfhydryl groups are still present in reasonable amount (50 to 60% of the original flour sulfhydryl content) in those doughs of poor mixing stability even after they have been severely overmixed. Consequently it appears that the sulfhydryl groups must not only be present in the dough but that they must also be accessible for reaction. The flours of good mixing stability may illustrate this condition; they continue to decrease in sulfhydryl content over a long period of time. In fact, the observations on the flour-water suspensions together with those on doughs suggest that the loss (presumably by oxidation) of sulfhydryl groups follows their participation in the changes involved in dough formation and development. If these changes include sulfhydryl-disulfide interchange, the sulfhydryl groups produced by the interchange would appear to be more susceptible to oxidation than those originally present.

Acknowledgment

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THE DISTRIBUTION OF THE AMINO ACIDS OF WHEAT IN COMMERCIAL MILL PRODUCTS¹

F. N. HEPBURN, W. K. CALHOUN, AND W. B. BRADLEY

ABSTRACT

The eighteen commonly occurring amino acids were determined by microbiological analysis in two blends of hard red spring and hard red winter wheats and in each of the final products produced by commercial milling. The summation of amino acid values obtained for the separate products, weighted by the proportion of the whole wheat represented in each product, yielded total values in good agreement with the results determined for the wheat.

Changes in the relative proportions of the amino acids showed the same trends in both sets of samples. Less lysine, glycine, arginine, alanine, and aspartic acid was found in the flour fractions and more in the offals than in the wheat proteins. Conversely, more proline, glutamic acid, and phenylalanine was found in the flour proteins and less in the offals than was found in the wheats. The proportions of the remaining amino acids were much less affected by milling.

The uniformity of amino acid concentrations within the flour fractions explains the previous observation that the amino acid distribution of flour proteins was independent of the degree of separation of the flour. It is postulated that the consistency of amino acid distribution in bread flour protein may be related to the selection and blending of wheats.

Previous work from this laboratory (2) indicated that although the concentrations of amino acids in flour differed from those of the wheats from which they were milled, the relative proportions contained in the proteins of the flours tended to be constant even though the flours which were analyzed ranged from 98.5 to 80% patent. The observed consistency of amino acid proportions of flour proteins could not be explained by previously published data, because the few studies on the amino acids of mill products have been limited either in the number of amino acids or in the number and type of products investigated. This present study was undertaken to determine quantitatively the distribution of amino acids among the products resulting from the commercial milling of wheat.

Materials and Methods

Sample Description. Samples of cleaned wheat and the products milled from them were obtained from two flour mills. Both wheats were blends of hard red spring and hard red winter wheats, typical

¹ Manuscript received May 5, 1960. Presented at the 45th annual meeting, Chicago, Ill., May 1960. Contribution from the American Institute of Baking, Chicago, Ill. This research supported by a grant from the Max C. Fleischmann Foundation.

of the type used for the production of baker's flour. From mill A the final products were: farina, flour (80.1% patent), first-clear flour, low grade flour, red dog, shorts, bran, and germ. In addition, samples were collected of the best- and poorest-quality flour streams. The extraction rate was reported to be 75.75%. The yield of the products (percent of the cleaned wheat) was determined by measuring the weights obtained after processing a known amount of wheat. From mill B the final products were: flour (95% patent), second-clear flour, red dog, shorts, bran, and germ. The extraction rate was approximately 68%. Values for yield constitute estimates furnished by the mill.

The samples of wheat, bran, and germ were ground in a hammer mill to pass a 0.024-in. screen. Nitrogen was determined by the Kjeldahl-Gunning procedure and moisture by the vacuum-oven method. Values for yield and for nitrogen are shown in Table I.

TABLE I DESCRIPTION OF MILL PRODUCTS (14% moisture basis)

MILL PRODUCT	Mrs	L A	Mn	L B
MILL PRODUCT	Nitrogen	Percent of Wheat	Nitrogen	Percen. of Whea
	%		%	
Farina	1.88	1.51		
Best flour stream	1.96			***
Patent flour	2.05	60.68	2.06	64.5
First-clear flour	2.60	11.14		
Second-clear flour			2.69	5.5
Low-grade flour	2.88	2.42	***	***
Poorest flour stream	3.02	b.		
Red dog	2.87	0.48	2.90	1.0
Shorts	2.86	9.13	2.82	15.0
Bran	2.54	14.57	2.79	12.0
Germ	3.98	0.07	3.78	2.0
Whole wheat	2.27	100.00	2.24	100.0

⁹ Constituent of patent flour. ^b Constituent of low-grade flour.

Analysis of Amino Acids. The 18 commonly occurring amino acids were determined by microbiological analysis in all mill products and in the original wheat samples using the procedures described previously by Hepburn et al. (2). The only modification was that Leuconostoc citrovorum 8081 was utilized for the analysis of cystine. This organism has been found to give a more consistent response with the short-term hydrolysates used for cystine than does Leuconostoc mesenteroides. Preliminary investigations showed that the removal of fat from germ and bran was not necessary for the determination of amino acids in these products. Analysis of solvent-extracted samples gave values identical to those obtained without such treatment.

Results and Discussion

All amino acids were determined at least twice in each of duplicate hydrolysates of each product. Average results of analysis of samples from mill A are given in Table II and those of mill B in Table III. Values have been calculated to the 16% nitrogen basis so that changes in protein composition can be followed.

Comparison of Results between Mills. Comparison of the data in Tables II and III reveals no pronounced differences between the two samples of wheat or between the two patent flours. Values for these products are in excellent agreement with those previously reported (2), except that the cystine content of each was found to be lower. It is not known whether this difference can be attributed to the samples or to the use of the different assay organism. In general, the data for the flours are also in agreement with those reported by McDermott and Pace (4).

Values for the offals may not be comparable between mills because of differences in milling procedures. Differences in amino acid contents may reflect the extent of separation of bran from germ and the relative amounts of fragments of these products which are contained in the red dog and shorts fractions.

Comparison of Products. Changes in the relative proportions of the amino acids showed the same trends in the samples from both mills. Less lysine, glycine, arginine, alanine, and aspartic acid was found in the flour fractions, and more in the offals than in the wheat proteins. Conversely, more proline, glutamic acid, and phenylalanine was found in the flour proteins and less in the offals than was found in the wheat. The proportions of the remaining amino acids were much less affected by milling. These findings for wheat and flour are in general agreement with those obtained by chromatographic analysis of Manitoba wheat and flour as reported by Nunnikhoven and Bigwood (6), but these workers did not investigate other milling products. Earlier values reported by Barton-Wright and Moran (1) on the distribution of amino acids in different fractions of wheat are difficult to compare with the data presented here because only 11 amino acids were studied, and of these, a number are at such wide variance with those cited above for wheat and flour as to suggest analytical differences rather than product differences.

Of greatest interest to the purpose of this paper is the distribution of amino acids in the proteins of the flour fractions. It can be noted

TABLE II
CONCENTRATION OF AMINO ACIDS IN PRODUCTS OF MILL A
(g. per 16 g. nitrogen)

	ALANINE	увсиние	Азгадата Аста	Стапия	Съптажие Асто	Стасия	яміцітвіН	проспессия	PERCURE	Гления	зигионтяК	Ривичельния	Рвосиив	SERINE	зигиозинТ	иличотчяТ	TYROSINE	WALLAW
Farina	2.73	3.77	4.01	1.76	33.4	3.03	1.92	4.01	6.72	2.01	1.73	4.91	11.68	5.36	2.70	1.04	3.35	4.31
Best flour stream	2.65	3.65	3.86	1.80	34.8	2.90	16.1	3.94	89.9	1.94	1.68	4.88	11.63	5.34	2.59	1.02	3.31	4.27
Patent flour	2.67	3.73	3.90	1.76	33.7	2.96	1.92	3.91	6.63	1.97	1.73	4.77	11.69	5.40	2.64	0.92	3.27	4.32
First-clear flour	2.75	3.87	3.86	1,85	34.7	3,25	2.06	4.02	6.59	1.94	1.71	5.04	11.75	5.32	2.73	1.01	3.35	4.44
Low-grade flour	3.10	4.68	4.50	1.67	29.6	3.70	2.14	3.72	6.33	2.54	1.67	4.64	10.16	5.19	2.76	1.01	3.20	4.45
Poorest flour stream	3.79	5.91	5.55	151	25.8	4.37	2.27	3.65	6.16	3.17	1.74	4.25	8.58	5.07	2.89	1.07	3.08	4.73
Red dog	4.70	6.84	6.76	1.40	17.9	4.98	2.22	3.42	5.77	4.13	1.70	3.55	6.30	4.85	3.11	1.25	2.85	4.91
Shorts	4.74	6.85	6.95	1.36	9.91	5.33	2.20	3.31	5.64	4.18	1.62	3.44	6.03	4.69	3.03	1.29	2.85	4.84
Bran	4.65	09'9	6.64	1.52	16.2	5.12	2.55	3.29	5.51	3.77	1.48	3.58	6.11	4.58	2.86	1.58	2.82	4.69
Germ	5.23	88.9	7.48	1.04	14.0	5.22	2.26	3.48	5.75	5.28	1.91	3.38	5.03	4.60	3.42	96.0	2.85	4.90
Whole wheat	3.37	4.71	4.85	1.80	29.3	3.94	2.19	3.78	6.52	2.67	1.74	4.43	9.94	5.29	2.76	1.13	3.19	4.69
Weighted sum of products*	3.18	4.48	4.61	1.70	29.6	3.56	2.01	3.77	6.34	2.46	1.69	4.49	10.31	5.19	2.72	1.07	3.16	4.43

a Sum of amino acid values of products multiplied by percent of product milled from whole wheat.

CONCENTRATION OF AMINO ACIDS IN PRODUCTS OF MILL B

							18. Pc	9 21	(8: per to 8: mitogen)	(ma								
	ALANINE	АнгизаА	Авеатіс Асів	випетЭ	GLUTAMIC ACID	егисия	акидизан	INDURENCINE	Генсия	руман	METHIONINE	PHENTLALATINE	PROLINE	anmag	3NINOTHH I	MANAGISTAL	TYROSTA	Anting
Patent flour	2.72	3.97	4.06	1.70	35.5	9.19	1.80	4.05	06.9	2.05	1.70	5.17	11.70	5.52	2.86	1.08	9 8	464
Second-clear		À															00.0	Tor
. flour	2.95	4.43	4.43	1.69	35.1	3.41	1.87	4.04	6.84	2.32	1.71	5.00	11.53	5.54	2.90	1.04	14.8	4.65
Red dog	4.50	6.50	6.13	1.55	22.3	4.37	2.01	3.58	6.15	3.67	1.68	4.00	7.61	4.91	3.21	1.21	2.98	4.89
Shorts	4.92	16.9	6.59	1.44	9'81	4.90	2.04	3.36	5.86	4.04	1.56	3.70	99'9	4.83	3.22	1.33	2.85	4.82
Bran	4.55	99.9	6.15	1.45	19.7	4.74	1.94	3.41	5.78	3.84	1.51	3.76	6.92	4.66	3.05	1.36	9.89	4.70
Germ	5.08	7.04	7.19	1.19	17.3	4.94	2.08	3.28	5.72	4.78	1.73	3.68	60.9	4.60	3.44	1.10	9.84	4 91
Whole wheat	3.34	4.88	5.09	1.66	31.6	3.79	1.91	3.86	6.58	2.76	1.73	4.66	10.24	5.25	2.96	1.17	8.95	4.79
Weighted sum of products*	3.35	4.85	4.79	1.62	30.6	3.70	1.86	3,83	6.57	2.64	1.65	4.73	10.21	5.28	2.95	=	3.23	4.68

a Sum of amino acid values of products multiplied by percent of product milled from whole wheat.

in Table II that the amino acid concentrations are nearly constant for farina, patent flour, and the best-quality flour stream. Moreover, the values for the clear flour differ only slightly from those of patent flour. Because of the small differences in the amino acid composition of the flour fractions and because the low-quality fractions represent such a small proportion of the total flour, even extreme variations in the degree of separation would produce insignificant differences in the amino acid content of the patent flours produced. For example, from the lysine data (which show the greatest change in milling) and from the yield data it can be calculated that if the clear and low-grade flours were combined with the patent flour of mill A the lysine content would be increased to only 1.98% as compared to the value of 1.97% found for the patent flour. Similarly, inclusion of the second-clear flour with the 95% patent flour of mill B would yield a predicted lysine level of 2.04% as compared to the value of 2.02% found for the patent flour. This serves to explain the previous observation that the amino acid distribution of patent flours has been found to be nearly independent of the degree of separation of the flour.

This observation is at variance with the results of Nunnikhoven and Bigwood (6), which indicated greater loss of lysine in milling a 65% extraction flour than one of 75%. Calculations from their data yield protein values of 12.0, 11.9, and 11.5% (nitrogen × 5.7, 14%) moisture basis) for the wheat, 75% extraction flour, and 65% extraction flour, respectively. It should be noted that these authors have confused the term "percent patent" with percent extraction in comparing their results with those previously published by this laboratory (2); but this does not invalidate their findings. It is possible that the disparity is related to the difference in wheat source, the method of milling, or a combination of these factors.

Lawrence et al. (3) reported that an inverse relationship exists between the protein content of wheat and the concentration of lysine in the protein. McDermott and Pace (5) recently published evidence indicating that lysine, arginine, and, to a lesser extent, histidine showed the same inverse relationship to protein in three flours of different protein content. The data of Lawrence et al. (3) and unpublished data of this laboratory suggest that the inverse relationship between lysine level and protein level is pronounced in samples of low protein content and tends to disappear at high levels of protein. Most of the samples studied by this laboratory represent commercial blends of wheat intended for the production of bread flour and hence are generally high in protein. It is possible that

this alone accounts for the uniformity of the amino acid distribution in these samples; or it may be that the empirical blending of wheats to produce flour with desirable bread-baking qualities depends upon securing a blended protein having a fixed amino acid pattern.

Acknowledgment

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THE DISTRIBUTION OF THE VITAMINS OF WHEAT IN COMMERCIAL MILL PRODUCTS

W. K. CALHOUN, F. N. HEPBURN, AND W. B. BRADLEY1

ABSTRACT

Nine vitamins were determined in a commercial wheat blend (50% hard red spring and 50% hard red winter) and in the products resulting from its milling. Thiamine was assayed chemically. Riboflavin, niacin, pantothenic acid, folic acid, biotin, p-aminobenzoic acid, choline, and inositol were determined by microbiological methods. Choline was found not to be concentrated to any extent in any one product. The other vitamins followed, in general, the well-known distribution pattern of the enrichment vitamins (thiamine, riboflavin, and niacin), with the greatest proportion (50 to 90%) contained in bran and shorts. Comparison of vitamin content of patent flour, first-clear flour, and low-grade flour shows pronounced and progressive increases in all vitamins except choline with decreasing grades of flour.

Studies in this laboratory showed some procedures for liberating vitamins from wheat and wheat products to be inadequate. Methods

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adopted for analysis of wheat, flour, and bread were published by Calhoun et al. (5). It was of interest to see if these methods could be applied satisfactorily to the analysis of mill products, and to determine the percentage of the vitamins of whole wheat recovered in the products of commercial milling. As stated in the previous communication (5), the determination of vitamin B6 in wheat and wheat products presents special problems and is requiring extensive investigation. These studies will be presented separately.

Materials and Methods

Sample Description. The same lots of samples from Mill A described in the preceding paper by Hepburn et al. (8) in their studies of amino acids were analyzed for vitamins. Sample descriptions are repeated in Table I for convenience. The samples were stored at -20°F. (-28.9°C.) until needed.

TABLE I SAMPLE DESCRIPTION OF WHEAT BLEND AND OF THE PRODUCTS COMMERCIALLY MILLED FROM IT (14% moisture basis)

	(11/0 moisture ously)	
	PERCENT OF CLEANED WHEAT	PROTEIN (N × 5.7)
		%
Whole wheat a	100.0	12.9
Farina	1.51	10.7
Patent flour ^b	60.68	11.7
First-clear flour	11.14	14.8
Low-grade flour	2.42	16.4
Red dog	0.48	16.4
Shorts	9.13	16.3
Bran	14.57	14.5
Ce-m	0.07	22.7

 $^{\rm o}$ Blend of hard red spring and hard red winter wheats. Extraction rate 75.75%. b 80.1% patent.

Assay Methods. Niacin, pantothenic acid, folic acid, biotin, paminobenzoic acid, choline, and inositol were determined by microbiological methods previously described by Calhoun et al. (5). Thiamine was determined by the thiochrome procedure as revised by Bechtel and Hollenbeck (4).

The fluorometric method of analysis for riboflavin (2) was unsatisfactory for products which, upon hydrolysis, produced highly colored extracts. Attempts to correct for nonriboflavin fluorescence met with varying success. Results of analysis of such products often varied markedly from day to day. However, the microbiological method using the assay organism Lactobacillus casei (1) proved satisfactory for all products.

Repeatedly, tests in this laboratory have failed to demonstrate the superiority of digesting wheat and wheat products (except germ) for pantothenic acid determination with the pigeon liver-alkaline phosphatase extract of Neilands and Strong (13) over the use of Mylase P2 as suggested by Ives and Strong (11). In fact, approximately 10% higher values were obtained by the use of Mylase P. The pantothenate value obtained for germ was 10.4 y per g. after digestion with Mylase P (Table II) and 15.8 y per g, following digestion with the pigeon liver - alkaline phosphatase extract. Tests were also carried out with the pigeon liver extract from which most on the pantothenic acid had been removed by adsorption on activated Dowex 1 resin according to the method of Novelli and Schmetz (14) as modified by Toepfer et al. (15). Results obtained with this preparation were not different from those previously found with the untreated extract. Clegg (6) found this two-enzyme system liberated approximately 50% more pantothenic acid from wheat and flour than did treatment with takadiastase plus papain.

Two or more hydrolysates were prepared from each sample and either five or six levels of each hydrolysate were analyzed in duplicate. Values falling beyond $\pm 10\%$ of the mean were discarded.

Results and Discussion

The vitamin levels found in the wheat blend and in the mill products are given in Table II. The mill products are arranged in general order of decreasing content of vitamins. It is doubtful if meaningful detailed comparisons can be made between the values given in Table II and values found in the literature. Wheat blends are usually composed of several varieties, often grown under different environmental conditions. Storage times and conditions may vary widely. Also it is difficult to compare product values from different mills because yield values may not be identical. Hinton and co-workers, employing the technique of hand-dissecting wheat and analyzing the various anatomical regions for vitamins, have contributed much to present knowledge of the distribution of vitamins within the kernel. The results obtained in the present study can be related to their findings only in a general way. Where inferences can be drawn, as in case of thiamine (9), niacin (7), riboflavin, and pantothenic acid (10), good agreement was obtained when based upon available knowledge as to the kinds and relative amounts of tissues present in the products.

The literature is replete with values for thiamine, riboflavin, and niacin in wheat and in mill products. It is sufficient to note that values

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VITAMIN CONTENT OF PRODUCTS COMMERCIALY MILLED FROM A SINGLE WHEAT BLEND (14% moisture basis) TABLE II

The second secon									
23	VHOLE	Вваж	Samus	Res Doc	Свам	Low. Grade Flour	FIRST. CLEAR FLOUR	PATENT	Равича
	wie	2/18	2/18	2/8	2/18	2/8	2/8	2/8	2/18
		000	101	0 80	18.5	10.8	2.45	0.76	0.42
	5.93	0.23	13.4	8 99	4.87	1.24	0.48	0.32	0.28
	1.07	3.34	160.0	27.08	45.8	38.6	20.9	10.1	8.79
4	6.40	2007	0.001	18.9	10.4°	9.15	6.75	4.83	4.44
ic acid"	0.9	00.0	1 95	1 90	202	0.42	0.18	0.11	0.09
	0.50	0.68	0.850	0.950	0.174	0.108	0.042	0.014	0.010
Biotin p-Aminobenzoic acid	3.83	14.8	12.6	7.81	3.70	2.95	1.26	0.83	0.24
	mole	mele	mele	8/2m	mg/g	2/2w	2/314	2/200	3/8m
Choline ⁴ Inoxiol	1.63	154	1.76	1.74	2.65 8.52	3.41	1.51	1.61	1.63

a As thiamine hydrochloride, b As calcium pantothenate, e Pigoon liver — alkaline phosphatase digestion yielded 15.8y per g. As choline chloride.

PERCENTAGE OF THE TOTAL VITAMIN CONTENT OF WHEAT FOUND IN EACH COMMERCIALLY MILLED PRODUCT TABLE III

	Вели	SHORTS	PATENT FLOUR	Finst- Clean Flour	Low. Gasse Flour	Red Doc	Farina	Ски	TOTAL
	28	8	PS.	R	N.	N.	1º	18	1/2
Yield from wheat	14.57	9.13	89.09	11.14	2.42	0.48	1.51	0.07	100.0
Choline	13.8	6.6	59.9	10.3	01	0.5	27	0.1	98.2
Pantothenic acid	52.3	22.3	26.9	. 6.9	2.0	8.0	9.0	0.1	9.111
Riboflavin	45.5	29.6	09.0	5.0	8.8	1.4	0.4	0.3	103.2
Thiamine	23.3	33.0	15.1	0.6	8.6	3.4	0.2	0.9	92.8
Folic acid	25.6	24.6	13.4	4.0	2.0	5.1	0.3	0.3	71.4
Niacin	71.1	26.8	11.2	4.3	1.7	0.7	0.2	0.1	116.1
Biotin	56.2	28.0	7.4	4.1	2.3	1.0	0.1	0.1	99.2
Inositol	62.0	31.3	6.4	4.0	2.6	2.1	0.1	0.5	107.8
p-Aminobenzoic acid	56.3	30.0	03.00	3.7	1.9	1.0	0.1	0.1	98.3

found in Table II agree broadly with those summarized from the literature by Bailey (3), and by Kent-Jones and Amos (12). Only fragmentary studies of the pantothenic acid, folic acid, biotin, p-aminobenzoic acid, choline, and inositol content of mill products have been reported. Some of these studies have been summarized (3,12). Where values are found, the samples are often inadequately described and differences between laboratories could be ascribed either to samples, methods of determination, or both. The authors failed to find complete studies of the content of these vitamins in the products of milling from a single wheat source.

Comparison of the vitamin content of patent flour, first-clear flour, and low-grade flour shows pronounced and progressive increases in all vitamins except choline. This is in contrast to the findings with amino acids (8), which exhibited little or no difference between flour products.

The percent of the vitamin content of whole wheat that appeared in each mill product (Table III) was calculated by multiplying the vield value (Table I) by the vitamin concentration (Table II) and dividing the resulting product by the vitamin content of whole wheat. The mill products are arranged in Table III in general order of decreasing contribution to the total vitamin contained in whole wheat. Patent flour's contribution of choline provided the major exception, since this vitamin was not concentrated in any one product by milling. For example, patent flour constituted 60.68% of the wheat and contained 59.9% of the total choline of that wheat. This relationship between yield of product and contribution of choline was found for the other products. The vitamins are listed in Table III according to decreasing amounts of whole wheat vitamin retained in patent flour. Thus, 59.9% of choline was retained, but only 5.2% of the p-aminobenzoic acid of wheat appeared in patent flour.

Bran and shorts contained, except in the case of choline, approximately 50 to 90% of the total vitamins present in the cleaned wheat. Except for niacin and p-aminobenzoic acid, the vitamin content of germ was higher than in whole wheat (Table II). However, when the amount of germ contained in wheat is considered, assuming 2 to 2.5% of the kernel is germ, the amounts of vitamins removed in this product by milling are far less than the total contained in bran or shorts.

The sums of vitamins found in the mill products (Table III) were within about 3% of the amount contained in whole wheat in case of choline, riboflavin, biotin, and p-aminobenzoic acid. Ninety-three

percent of the thiamine and 108% of the inositol appeared in the products. Less satisfactory, but acceptable recoveries of pantothenic acid (112%) and of niacin (116%) were obtained. Unaccountably, only 71% of the folic acid of wheat was found in the mill products.

It is hoped that the methods adopted for use in these studies will prove useful in future work designed to understand better the extent to which various factors influence the vitamin content of wheat and wheat products.

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A NOTE ON THE MODIFICATION OF THE FIVE-GRAM MILLING-QUALITY TEST AND THE FIVE-GRAM MICROMILL¹

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ABSTRACT

Elimination of the bran-weighing procedure and equipping a 5-gram micromill with a detachable hopper resulted in a 200% increase in the number of samples that can be evaluated for milling quality in a day, and significantly reduced operator fatigue.

The micromill used in Seeborg and Barmore's (1) micromilling test consisted of two breaks followed by a sifter with a No. 38 wire screen. The material remaining on this screen after milling and sifting was mainly bran and attached endosperm.

Visual comparison of this bran with that from varieties whose milling characteristics were well known showed that varieties with high flour yield, low flour ash content, rapid bolting properties, etc., had less endosperm attached to the bran than varieties with poor milling properties. Such good milling varieties generally yielded less than 1.65 g. of bran from 5 g. of grain.

While the majority of plant breeders' samples could be evaluated by the bran weighing procedure, there were always some exceptions such as: low bran weight but unacceptable cleanup, and acceptable cleanup but high bran weight.

These exceptions were attributed to the following cumulative fac-

- Varietal differences in the fracturing the endosperm underwent in milling, which was reflected in the varying amount of coarse, chunky endosperm remaining on the No. 38 wire sieve with the bran. (A coarser sieve was not used because an undesirable amount of bran would pass into the endosperm used for micro flour quality tests.)
- Varietal differences in the fracturing the bran underwent in milling, which was reflected in the varying amount of bran passing through the No. 38 wire sieve.

¹ Manuscript received October 15, 1959. Contribution from the Western Wheat Quality Laboratory, Department of Agricultural Chemistry, Washington Agricultural Experiment Stations; and Crope Research Division, Agricultural Research Service, U. S. Department of Agricultural Information Paper, Washington Agricultural Experiment Stations, Pullman.
² Respectively: Analytical Chemist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture; formerly Assistant Milling Technologist, Washington Agricultural Experiment Stations; and Chemist, Crops Research Division, Agricultural Research Service, U. S. Department of

Agriculture.

 Moisture levels above 9% in the untempered wheat could contribute to low bran weight, even though the bran cleanup was unacceptable.

Visual evaluation of the bran cleanup was, therefore, necessary to eliminate the breeding material with undesirable milling characteristics; moreover, it was considerably faster. This procedure has proved very satisfactory to the cooperating wheat breeders.

Elimination of the bran weighing has reduced the over-all time for the milling cycle by one-half and doubled the number of samples that can be analyzed in a day.

With the original feed hoppers (2) it was necessary to stop the micromill in order to load a new set of samples, because there was no valve to stop the grain from flowing into the rolls from the hoppers. The two operators were idle during the time that the rolls were operating, and this constituted one half of the total milling time. Inasmuch as there was sufficient time to load a new set of samples into the hoppers, it became apparent that the total milling time could be reduced by one-half if some way could be devised to do this while the rolls were in operation.

This need led to the design and construction of a detachable hopper (Figs. 1 and 2) equipped with a slide valve which is held closed

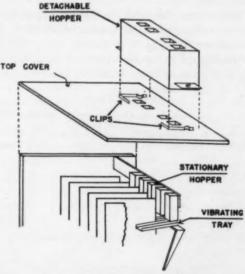


Fig. 1. Hopper in position on mill.

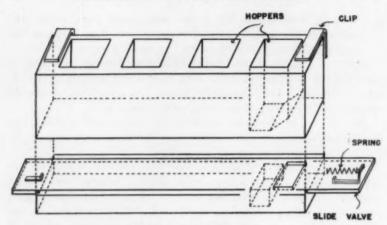


Fig. 2. Detachable hopper.

by a compression spring. The hopper is loaded with a new set of samples and clipped into position on the top cover of the mill above the stationary hoppers while samples are still going through the breaking operations. When a new milling cycle is started, all four compartments of the detachable hopper are emptied quickly and simultaneously by pushing the slide valve to the open position. The spring then closes the valve.

This new hopper, together with the elimination of bran weighing, has increased the testing capacity of the micromill 200%. In addition, it has significantly reduced operator fatigue, since the operator can remain seated during the entire operation instead of having to change from a sitting to a standing position several hundred times a day.

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DETERMINATION OF BREAD CRUMB COLOR AS RELATED TO THE COLOR OF FLOUR USED TO BAKE THE BREAD!

Y. Pomeranz²

ABSTRACT

Bread color was measured with the Kent-Iones & Martin Color Grader. using a slurry prepared by mixing bread crumb, corn starch, and water in a Waring Blendor. Determinations were made on crumb removed from the center of the loaf and slurried in a 1:1 ratio of starch to bread crumb, for 30 seconds. Delay in color measurement affected the results due to settling of bread crumb solids. The results were unaffected by variations in fermentation and proof time by loaf size and loaf form, and by addition of lactic acid. Storage at elevated temperatures resulted in darkening of bread crumb. The effect of starch employed in bread color determinations was established and a formula for calculating grade color of flour used in a bread under test was determined. The resulting method was applied to a number of flours of varying grade and the breads made from them. The procedure was found to measure that component of bread crumb which is independent of crumb grain and texture; this was shown to be highly correlated with the grade color of the flour from which the bread was baked.

The color of bread crumb depends on a number of factors, the most important of which are the color of flour from which the bread has been produced and the method of bread manufacture. The apparent bread color depends on crumb grain; thus Treloar et al. (17) and Graesser (5) have reported correlations between crumb color and texture scores. Properly fermented bread has a fine crumb with soft texture, good sheen, and bright color. The improved color is due to the finer cell walls surrounding small gas cells; this structure tends to reflect the light instead of absorbing it, thus looking much whiter than an improperly fermented bread baked from the same flour. Under normal storage conditions changes in the crumb color of wheat bread are negligible. On prolonged storage at elevated temperature a browning reaction takes place (15). Although the color of bread crumb is of great commercial importance, it has proved rather difficult to measure this property objectively. Markley and Bailey (12) stated that "A practical crumb scoring method which will remove most if not all the personal factors in judgment is very much needed," and that crumb color is one of the most important properties to be ascertained by the baking test.

Measurement of crumb color, employing color charts (14) or Mun-

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sell disks (1,2,6), is difficult and is influenced by pigment content and by crumb grain; hence the values obtained are of little value as an index of flour grade. Geddes (4) measured changes in bread crumb color by a rather complicated procedure, involving careful air-drying of bread crumb, grinding the crumb to a specified fineness, and measurement of the reflectance in comparison with a standard white surface employing a Beckman spectrophotometer, fitted with a reflectance attachment. Larsen et al. (10) measured changes in browning of bread crumb with a Hunter color and color difference meter. Wilcox (18) tested the utility of the Photoelectric reflectometer for color crumb measurement and found that the tristimulus values obtained by means of this instrument could be used in an objective differentiation between samples which were visually different. Szalai (16) studied light reflectance from bread slices, pressed bread samples, and frozen bread crumb; satisfactory reproducibility was obtained when measurements were made on vacuum-dried and ground bread crumb.

The Kent-Jones & Martin flour color grader has found a wide use in determination of flour color in cereal laboratories (8,9). The principle of the instrument is the measurement of light reflected by a flour slurry. The instrument uses a filter with maximum transmittance at 530 m μ ; the readings reflect primarily the amount of bran present, and are only slightly affected by the color or fineness of bran particles, carotenoid pigments, or by flour particle size. Several workers have found that the reflecting power of flour should be measured at several wave lengths (3,7,13). Though the Kent-Jones & Martin flour color grader measures only one color attribute, it determines the major factor in judging of flour grade: the brightness or dullness which is correlated with the flour extraction rate.

Mackinney (11), while discussing flour color measurement, mentioned the necessity of formulating a relationship between readings of flour color measurement and crumb color of the loaf. In dealing with flours of medium to low quality which produce small loaves with fairly poor texture, grain and texture tend to mask differences in flour grade color. The present study has been undertaken to develop a method for measuring, by means of the Kent-Jones & Martin color grader, that component of crumb color which is independent of crumb grain and texture, and to determine its relation to the grade color of the flour. The method involves mixing corn starch with bread crumb and water to form a slurry, the color of which is measured in the color grader. The influence of a number of variables in analytical procedure, bread storage conditions, fermentation and proof time on the bread color readings was studied and the resulting method

applied to a number of flours of varying grade and the breads baked from them.

Materials and Methods

Flour Samples. Untreated flours of different extraction from 23 commercial mills were used. The flours were milled from a medium-strength wheat grist (about 11.5 to 12% protein)—a mixture of hard red winter, soft red winter, Italian, and Turkish as well as local soft wheats. The range of flour extraction of samples used in this study was between 52 and 95% (calculated as percentage of total products obtained on milling); their ash content varied between 0.55 and 1.35%.

Starch Samples. Commercial corn starch from five sources was used. The grade color of the starches used ranged between -1.2 and +3.0. The grade color of starches employed, except for those used in a series designed to determine the influence of starch color employed on bread color readings, was -0.6 to -0.3.

Analytical Methods. Flour color was determined according to the procedure outlined by the manufacturer, involving measurement of light reflected from the surface of a paste prepared from 30 g. of flour and 50 ml. of water. Starch color was measured on a paste prepared by mixing 40 g. of starch with 40 ml. of water. Unless stated otherwise, bread color was determined by the following procedure: a slurry of bread crumb was prepared from 40 g. of shredded, crustfree bread crumb, taken from the interior part of the bread about 48 hours after baking, and mixed 30 seconds with 40 g. of corn starch and 85 ml. of water in a Waring Blendor. The slurry was poured into the cell of the Kent-Jones & Martin color grader and the color measured. The usual precautions observed during measurement of flour color with the instrument-water temperature, paste consistency, etc. - were also observed in testing bread-crumb color. Minor changes in the ratio of water to solids caused no significant variations in color determinations.

The effect of a number of variables in analytical procedure was studied. These include: delay in color measurement, effect of location in the loaf from which the bread crumb has been taken, effect of starch color, slurry mixing time, and effect of the ratio bread crumb to starch used in making the slurry. Additionally, the effect of variations in bread-baking procedure (fermentation and proof time, loaf size and form, addition of lactic acid) and storage conditions on bread-crumb color has been studied.

TABLE I Bread-Baking Method

	WHITE BREAD	DARK BREAD
Flour (g.)	1000.0	1000.0
Salt (g.)	13.0	20.0
Active dry yeast (g.)	15.0	7.5
Dispersed for 15 minutes in water (ml.)	90.0	50.0
Dissolved with sucrose (g.)	36.0	2.0
Lactic acid, 20% (ml.)		25.0
Calcium propionate (g.)	2.0	
Malt extract (g.)	2.5	
Potassium bromate (p.p.m.)	20.0	
Water	variable	variable
Bulk fermentation (minutes)	about 110	about 75
Proof (minutes)	about 75	about 70
Dough temperature (°C.)	30	30
Fermentation temperature (°C.)	30	30
Baking time (minutes)	about 50	about 50
Baking temperature (°C.)	220	220

Bread-Baking Methods. Unless stated otherwise, the bread tested was baked in a testing bakery by the method outlined in Table I.

White bread was baked from flours of grade color below 9.0; for dark bread, darker flour was used. The loaves were scaled (at about 580 g.) so as to weigh 500 g. after baking.

Results and Discussion

Variables Affecting Bread-Crumb Color Determination. The standard error of a grade color determination of the bread crumb was 0.161, based on duplicate analyses of nine typical samples. Increasing the mixing time from 30 to 60 seconds in preparing the slurry in the Waring Blendor had no effect on grade color readings, and a mixing time of 30 seconds was normally employed. Mixing time beyond 30 seconds sometimes gave a stiff slurry, especially in bread made from high-extraction flour and impaired paste consistency, causing difficulties in pouring the slurry into the cell. There is, however, no danger in increasing the mixing time to one minute, if this should be necessary to obtain a slurry of uniform composition.

Though there was a linear relation between the ratio of starch solids to bread crumb solids and grade color (Fig. 1), tests were made with a 1:1 ratio as a matter of convenience in order to be able to measure grade color of various sturries within the working range of the instrument.

The grade color of the bread crumbs, in Fig. 1, was 4.3 and 10.5 (in samples A and B respectively) when tested with a slurry containing a 1:1 ratio of starch to bread crumb.

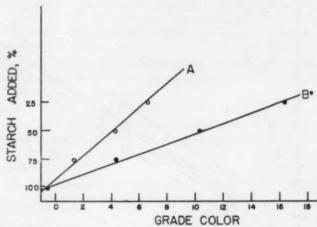


Fig. 1. Effect of various amounts of starch (as percent of total solid ingredients) on grade color.

Readings of color grade were made immediately after completion of mixing, because delay in the color measurement had a significant effect on results, caused by settling of bread solids (see table below).

Sample No.		Effect of Delay (m leasurement on B		
	0	5	15	30
1 2	5.1 8.3	5.4 8.8	5.6 9.0	5.8 9.3

Bread crumb was generally removed from the interior of the loaf. The effect of including also some part of the exterior (care being taken not to include crust) is given in Table II.

The results show that the place from which the bread crumb was removed had a significant effect (5% level) on grade color. Subse-

TABLE II
EFFECT OF PLACE FROM WHICH BREAD CRUMB WAS REMOVED ON GRADE COLOR

SAMPLE No.	INTERIOR ONLY	Extensor Incluses
1	7.5	7.5
2	7.5	7.7
3	9.4	9.4
4	10.2	10.4
5	10.7	11.1
6	11.3	11.4
7	11.4	11.4

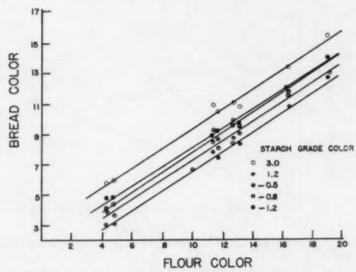


Fig. 2. Effect of starch color on relation between flour color and bread color.

quently, only the interior crumb was used for color measurement.

Relation between Bread Color and Flour Color. Figure 2 shows the relation between flour color and bread color, with the use of five different starches in preparing the slurry. Bread was baked from 16 flours, ranging in grade color from 4.3 to 19.0, and duplicate crumb color determinations were made using each of the starches.

For the five corn starches used, the following formulas for linear regression lines were obtained.

Starch Color	Linear Regression*
-1.2	y = 0.652x + 0.143
-0.8	y = 0.642x + 0.892
-0.5	y = 0.654x + 1.306
+1.2	y = 0.618x + 1.895
+3.0	y = 0.647x + 2.954

[&]quot; y = Grade color of bread slurry; x = grade color of flour.

The multiple regression was calculated: Y = 0.640X + 0.554Z + 1.202, where Y = grade color of bread slurry, X = grade color of flour, and Z = grade color of starch. The flour and bread color had a high over-all positive linear correlation: $r = +0.948^{xxx}$ from a total of 72 pairs of observations, using five different starches. The correlation between flour color and bread color was increased to a partial correlation of $r_{yx.x} = 0.987^{xxx}$, when starch color was held fixed.

The multiple correlation between the observed flour color and those calculated from the multiple regression equation given previously was 0.988xxx, which is larger than either simple correlation with bread color.

These data enable one to calculate from bread color data the grade color of the flour from which the tested bread was baked, provided the color of starch is known.

Effect of Variations in Bread-Baking Procedure and Bread Storage on Bread Color. In a series of experiments it was found the neither the loaf size (500 to 1,000 g.) nor the form (panned or open hearth; oblong or round) had any significant (at 5% level) effect on color of the crumb.

Table III shows the grade colors of bread crumbs baked from doughs fermented and proofed for various times.

TABLE III
EFFECT OF FERMENTATION AND PROOF TIME ON GRADE COLOR

	FERMENTATION TIME	Proor	LOAF VOLUMER	COLOR RANKING (VISUAL) ⁸	COLOR
	hours	minutes	cc		
Dark bread	1	60	1.175	3	10.3
	2	75	1,300	2	10.4
	3	90	1,300	1	10.1
	1	60	1,300	3	10.9
	2	75	1,375	2	11.0
	3	90	1,475	1	11.2
White bread	1	60	1,500	3	4.3
	2	75	1,600	1	4.0
	3	90	1,225	2	4.4
	1	60	1,575	3	4.6
	2	75	1.675	1	4.5
	3	90	1.225	2	4.6

a Samples were sorted into three classes and labeled 1, 2, and 3, in order of decreasing color preference.

The results show no significant differences in grade color readings, in spite of rather wide variations in fermentation and proof times. When the same crumbs were scored visually, the differences in color were quite pronounced. This is in agreement with reported observations that variations in fermentation and proofing have a definite effect on visual appearance of loaf crumb color. These differences could not be measured by the proposed method after the crumb texture was destroyed by slurrying.

The addition of lactic acid (at levels varying between 0.2% and 0.8% on flour basis) had no significant effect on the color of the

crumb, as measured by the proposed method.

The determinations of grade color were made, generally, 48 hours after baking. Bread stored at room temperature showed no significant change in crumb color of bread within 48 hours.

Storing bread for 1 week at 28° to 30°C. affected the grade color of crumb; pronounced differences (significant at 5% level) were determined on storage at elevated temperatures after 48 hours.

	Crum	b Color of B	read Stored at	28° to 30° C.
After 48 hours	3.4	6.7		0.8 12.7
After 1 week	3.6	7.3		1.6 14.5
	Cri	imb Color o	Bread Stored	for 48 Hours
At 20°-30°C.	2.2	2.7	9.9	
At 65°-68°C.	3.8	4.9	11.2	

These results show that the method does detect changes in color due to bread crumb browning caused by either elevated temperatures or prolonged storage.

While under normal conditions the color of flour determines to a very large extent the color of bread crumb, several additional factors may influence the visual perception of bread color by the consumer. Though the effect of browning seems to be of rather minor importance (and is measured by the proposed method), texture has a definite effect on bread color score as judged by visual comparison of crumbs. By slurrying the bread, the differences in color due to these texture differences are avoided. The method seems attractive because of its simplicity and rapidity. It enables one to estimate the degree of extraction of the flour used in the bread, and to use a common basis for estimating color in both flour and bread.

Acknowledgment-

The author wishes to acknowledge his indebtedness to John A. Johnson for his valuable suggestions and counsel during the preparation of the manuscript.

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ERRATUM

Cereal Chemistry, Vol. 37, No. 1 (January, 1960)

PAGE 92, S. R. ERLANDER:

The editors regret that for reference 16 the given name was wrong: For DOROTHEA J. MANNERS read D. J. MANNERS (for DAVID J.).

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Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, of fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

Cereal Chemistry gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

The papers must be written in English and must be clear, concise, and styled for Cereal Chemistry.

Manuscripts for publication, and correspondence directly pertaining to them, should be sent to the Editor-in-Chief, CEREAL CHEMISTRY, 1955 University Ave., St. Paul 4, Minn. All other correspondence, including circulation, advertising, or other business, should be directed to the Managing Editor at the above address.

Manuscripts of published papers will be kept on file for one year. After that time they will be destroyed unless other instructions have been received from the author. Original graphs, etc., and negatives of all illustrations are returned to the author immediately upon publication.

SUGGESTIONS TO AUTHORS

General. Authors will find the last volume of Cereal Chemistry a useful guide to acceptable arrangement and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (Trans. Am. Assoc. Cereal Chemists 6:1-22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 81/2 by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Editorial Style. AACC publications are edited in accordance with A Manual of Style, University of Chicago Press, and Webster's Dictionary. A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°-10°C.). Place 0 before the decimal point for correlation coefficients (r = 0.95). Use \bullet to mark statistics that exceed the 5% level and $\bullet \bullet$ for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., A/(B+C). Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. The term extensigraph is to be used exclusively in papers which present experimental data obtained with either the Brabender Extensograph® (manufactured by the Brabender Corporation, Rochelle Park, N.J.,) or the Brabender Extensigraph (manufactured by C. W. Brabender Instruments, Inc., South Hackensack, N.J.). When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

^{*} Registered U.S. Patent Office.

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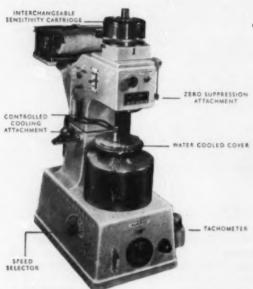
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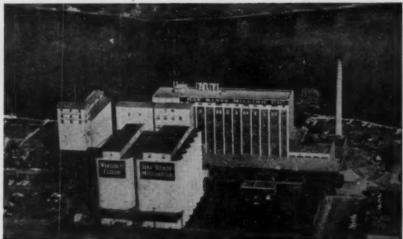
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